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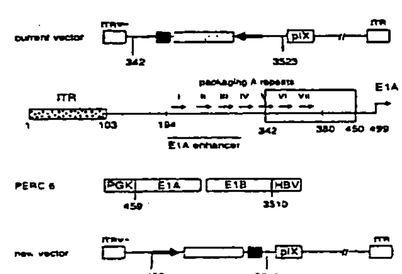
(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovector backbone in the generation of the new vector.

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replication-defective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. $CD8^+$ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive $CD8^+$ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from $CD4^+$ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.,* Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag, pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH_2-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication –defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a);and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective. They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BglII* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BglII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH_2-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of $CD3^+$ cells that were $CD8^+γIFN^+$ and $CD4^+γIFN^+$, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic seqeunces, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 μg and 200 μg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH_2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6® cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6®. Both these cell lines express the adenoviral E1 gene product. PER.C6® is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6®, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM $MgCl_2$; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM $MgCl_2$, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of $1x10^7$ to $1x10^{12}$ particles and preferably about $1x10^{10}$ to $1x10^{11}$ particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *Msc*1 site of the hCMV promoter and a 3' primer (designed to contain the B*gl*II recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *Msc*1 and B*gl*II. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *Msc*1 and *Bgl*II digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *Bgl*II digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *Bgl*II site. Colonies were screened using *Sma*1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, $(GT)_n$, and $(T)_n$; respectively) underlined:
AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	µg gag/10e6 COS cells/5µg DNA/48 hr
HIVFL-gagPR9901[a]	10.8
PVIJns-hCMV-FLgag-bGHpA[b]	16.6
pV1Jns-hCMV-FLgag-SPA[b,c]	12.0

[a] GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

[b] New plasmid constructions that have the intron A portion removed from the hCMV promoter.

[c] In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 µg and 200 µg.

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA[a] Promoter/terminator	Dose, ug[b]	Anti-p24 Titers (3 Wk PD1)[c]			SFC/10^6 Cells (4 Wk PD1)[d]		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV-FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV-FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

[a]in PBS

[b]i.m. Injections into both quads, 50 µL per quad

[c]n=10;GMT, geometric mean titer; SE, standard. error

[d]n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector -"MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

(1) The left ITR region was extended to include the *Pac*1 site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.

(2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.

(3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac*1 and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*1 linearized pAdHVO (E3- adenovector) or *Cla*1 linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI , BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and subsequently labeled with [^{33}P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and then labeled with [^{33}P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *Msc*1 overnight and then digested with *Sfi*1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*1. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH_20. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was *Pac*1 to release the vector backbone and 3.3 μg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [^{33}P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac*1/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10^6 cells/ml), Viability (%) Infection	Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^8 TCID$_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.86, 64%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.06, 66%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.86 2.60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	46	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 68%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10^6 cells/ml), Viability (%) Infection	Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^8 TCID$_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1.55, 86%	1.26, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 86%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

MRKAd5gag(E3-)

	Xv (10^6 cells/ml), Viability (%) Infection	Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^9 $TCID_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.86 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors[a]	µg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag[b]	1.40
Clinical lot Ad5gag[c]	1.28
Research lot Ad5gag[d]	1.32
MCMVFL-gagbGHpA[e]	0.42

[a] A_{260nm} absorbance readings taken for viral particle determinations.
[b] MRKAd5gag was produced in serum free conditions and purified at P5.
[c] Clinical lot# Ad5gagFN0001
[d] Research Ad5FLgag lot# 6399
[e] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	[a]MRKAd5gag	10^7	25600	5877	4780
2	"	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	"	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	"	10^9	310419	99181	75165
7	[b]mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	"	10^9	941014	239068	190636
9	[c]hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10	"	10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12	"	10^7	14703	5274	3882
13	"	10^8	58813	14942	11915
14	"	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16	"	10^7	4222	3405	1138
17	"	10^8	19401	3939	3274
18	"	10^9	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal
P.I.s: Youil, Chen, Casimiro
Vaccination: T. Toner, Q. Su
Assay: M. Chen

[a]The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).
[b]The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) ws used here.
[c]This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag[a], 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag[b], Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
[a]MRKAd5gag (hCMV, bGHpA, E3+)								
[b]original Ad5gag vector (hCMV/Intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of $CD4^+$ T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media[a]	Gag H[b]	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag	97N010	6	89	0	395	0	1058	0	1174	3	775	4	1074
	10^11 vp	97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag	97N120	5	275	1	249	4	141	4	119	9	206	4	219
	10^9 vp	97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot	97X001	0	261	1	485	0	817	0	1220b	1	894	0	1858
	10^11 vp	97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot	97N020	3	30	1	101	0	66	0	36	0	26	0	41
	10^9 vp	97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Naive	96R041	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	19	15	10	15	24	9

Based on either 4x10^5 or 2x10^5 cells per well (depending on spot density)
ND, not determined
[a]mock or no peptide control
[b]Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGA GTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

wt aa	aa residue	mutant aa	enzyme function
Asp	112	Ala	RT
Asp	187	Ala	RT
Asp	188	Ala	RT
Asp	445	Ala	RNase H
Glu	480	Ala	RNase H
Asp	500	Ala	RNase H
Asp	626	Ala	IN
Asp	678	Ala	IN
Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:3).

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.* 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH_2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

```
GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).
```

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

```
Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
```

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

```
CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID N0:11).
```

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).
```

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC
(SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *BglII* site. The clones were checked for the correct orientation of the gene by using restriction enzymes *DraIII/Not1*. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac1* and *Bst1107* I (or its isoschizomer, *BstZ107* I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla1* digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *PacI* (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ –60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac*1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ –60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA AT<u>G CGG CCG C</u>CA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II)Reverse: 5'-AAG GA<u>A GAT CT</u>A CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA AT<u>G GCG CGC C</u>AT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GA<u>A GAT CT</u>A CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc*1 and *Bgl*11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc*1/*Bgl*11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl*11 and the gag reporter gene (*Bgl*11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*1 and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgC12, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM $MgCl_2$, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μL of 1 μg /mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μL of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μL 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μL of 0.5M H_2SO_4 per well. OD_{492} readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD_{492} (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at $5x10^6$/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology.* John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples ($4\text{-}5x10^5$ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of $CD4^+$-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of $CD8^+$-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 ($CD8^+$ T cell epitope) or aa81-100 ($CD4^+$) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO_2, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10^6 cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN_3) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific $CD4^+$ and $CD8^+$ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers[a]			SFC/10^6 cells[c]		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2	310419	301785	153020	1(1)	75(4)	2313(67)
			1	919	372	265	1(1)	72(9)	533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2	1638400[b]	0	0	2(2)	114(9)	2063(182)
			1	713155	528520	303555	1(1)	48(7)	733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419	386218	172097	0(0)	223(7)	2607(27)
			1	6400	14013	4393	10(8)	141(21)	409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1638400[b]	0	0	1(1)	160(13)	2385(11)
			1	1241675[b]	396725	300661	0(0)	39(13)	833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

[a]GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean
[b]Near or at the upper limit of the serial dilution; hence, could be greater than this value
[c]No. of Spot-forming Cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers[a]			SFC/10^6 cells[b]		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2	174	70	50	1(1)	23(1)	1(1)
			1	132	42	32	0(0)	0(0)	0(0)
2	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174	70	50	0(0)	61(7)	4(2)
			1	132	42	32	1(1)	62(7)	3(1)
3	MRKAd5mCMVFLnef (E3+)	10^7 vp	2	132	42	32	3(1)	15(5)	5(2)
			1	115	46	33	3(2)	3(2)	4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2	132	42	32	4(2)	83(13)	5(1)
			1	132	42	32	2(1)	29(2)	4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132	42	32	3(2)	14(2)	5(1)
			1	100	0	0	3(1)	13(4)	10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2	230	170	98	3(2)	145(29)	4(0)
			1	115	46	33	7(1)	151(14)	10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	26(3)

[a]GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean
[b]No. of spot-forming cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IApol(E3+) 10^11 vp	99C100	1	0	0	1	38	31	0	52	146	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+) 10^9 vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	192	4	36	156	5	38	106
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IApol(E3-) 10^11 vp	99D239	5	2	2	20	82	172	1	66	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	236	1	24	264
MRKAd5hCMV-IApol(E3-) 10^9 vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Naive	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10^11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10^9 vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10^11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10^9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10^{10} vp/ml culture)	AEX Titer (10^4 vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%) Infection	Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%) Infection	Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10^6 cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10^{10} vp/ml culture	10^4 vp/cell	Ratio	10^{10} vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2×10^6 cells/ml. Cells were grown until they reached a cell concentration of approximately 1×10^6 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi ($1x10^{13}$ vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi ($1x10^{11}$ IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$-biased or $CD8^+$-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly $CD8^+$-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

Number of SFC/million PBMCs

Grp#	Priming T=0, 4, 8 wks	Boost T=26 wks	Monk#	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1	DNA/5 mgs	MRKAd5gag(E3+)	CB5H	NA	NA	3	35	15	71	4	224	8	115	6	85	19	956	0	316
	PBS	10^7 vp	CC6X	0	0	0	15	0	46	0	58	0	76	0	35	3	1705	1	755
	(D101)		AW3G	5	11	0	36	3	51	3	48	2	89	8	65	10	989	0	395
2	DNA/5mgs +	MRKAd5gag(E3+)	CC1C	0	4	1	60	0	111	5	270	4	280	8	232	3	959	19	1345
	CRL1005/45mgs	10^7 vp	CC1K	4	0	1	101	0	254	0	791	5	452	0	321	0	1916	1	1099
			AW3P	9	8	1	10	4	71	4	154	8	104	5	85	11	836	6	241
			CB5F	NA	NA	0	31	0	288	0	530	19	374	9	251	8	1549	20	1734
			AK6B	9	12	4	36	1	119	0	439	0	425	0	316	4	1229	5	1354
3	DNA/5 mgs+	MRKAd5gag(E3+)	AW20	10	4	1	59	5	264	19	425	6	105	9	205	18	565	8	404
	CRL1005/7.5 mgs + 0.6 mM BAK	10^7 vp	CA4R	1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
			CB58	8	6	0	6	3	119	0	274	6	282	1	208	0	636	1	828
			CB5W	4	3	0	26	1	91	0	139	0	164	1	62	5	543	1	349
			CB7D	1	0	0	136	0	316	1	609	5	626	1	759	0	2278	4	1831
4	none	None	98D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique Bam*HI* site. This fragment was digested with BstE*II* and Bam*HI*. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the Pst*I*-BstE*II* gag digestion fragment, the BstE*II*/BamH*I* digested PCR product and long Pst*I*/BamH*I* V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the Bgl*II* fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10^10 vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10^8 vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10^10 vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10^8 vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10^10 vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10^8 vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10^10 vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10^8 vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10^10 vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10^8 vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

:

1. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and

b) a gene encoding an HIV protein or immunologically relevant modification thereof.

2. A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.

3. A vector in accordance with claim 1 further comprising nucleotides corresponding to between from about base pair 3511 to about 3524 to about base pair 5798 of a wildtype adenovirus genome.

4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.

5. A vector in accordance with claim 4 which is deleted of base pairs 451-3510.

6. A vector in accordance with claim 1 which is at least partially deleted in E3.

7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6® cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) SEQ ID NO: 29;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6® cell.

62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

a) gag, pol, and nef, expressed independently from three individual vectors;

b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;

d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;

e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;

f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;

g) gag and pol, expressed independently from two individual vectors;

h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

i) pol and nef, expressed independently from two individual vectors;

j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

k) nef and gag, expressed independently from two individual vectors;

l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to a single promoter; and the encoding nucleic acid sequences operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

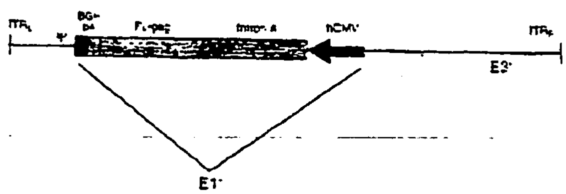

Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg
caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc
ctgctggagacctctgaggggtgcaggcagatcctgggccagctccagccctccctgcaaacaggctctgagg
agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc
acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag
gccatctcccccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc
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ccaggagcagattggctggatgaccaacaacccccccatccctgtgggggaaatctacaagaggtggatcat
cctgggcctgaacaagattgtgaggatgtactcccccacctccatcctggacatcaggcagggccccaaggag
cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact
ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg
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gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag
gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga
agggctgctggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaggccaacttcctg
ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct
cccgaggagtccttcaggtttggggaggagaagaccacccccagccagaagcaggagcccattgacaagg
agctgtacccccctggcctccctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca
gat (SEQ ID NO: 29)

Figure 2

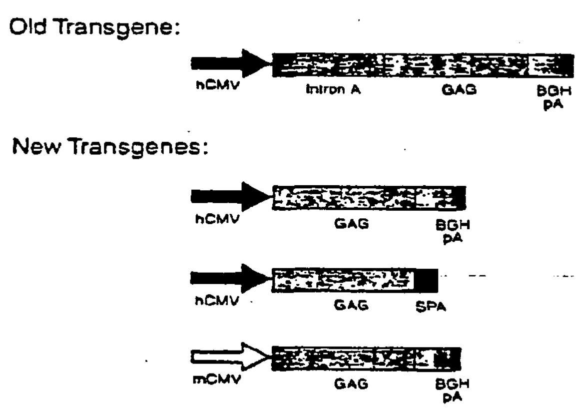


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

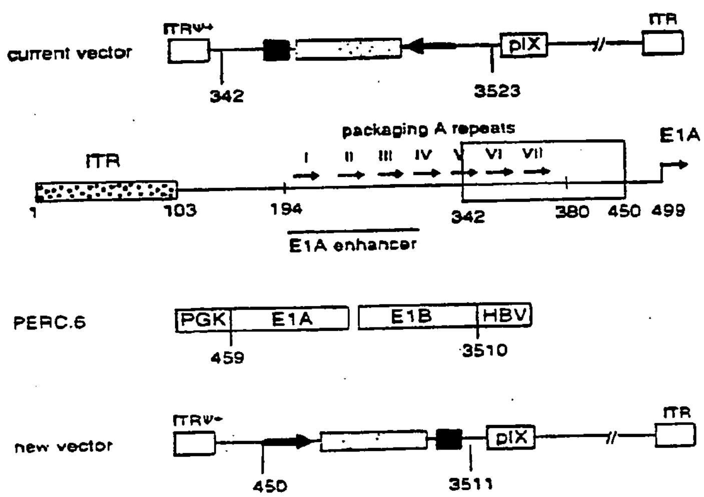


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

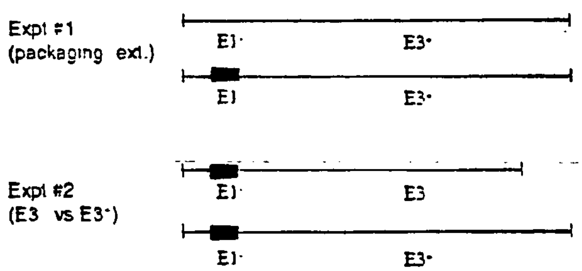


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (**Expt #1**) and analysis of the effects of the E3 gene on viral growth (**Expt. #2**). The red bars denote the region of modifications made to the E1 deletion.

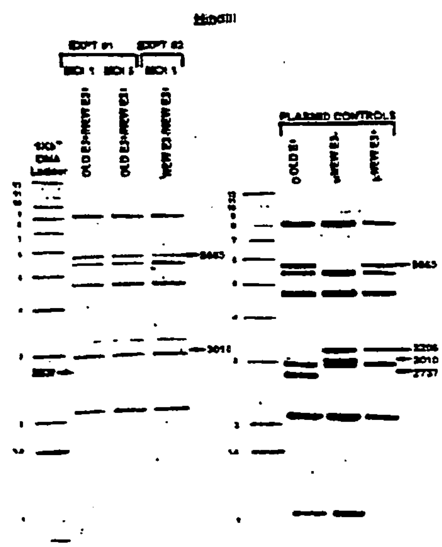


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (**expts. #1** and **#2**) as detailed in the text.

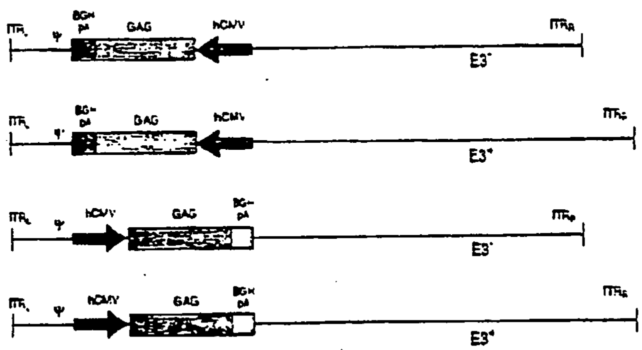


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

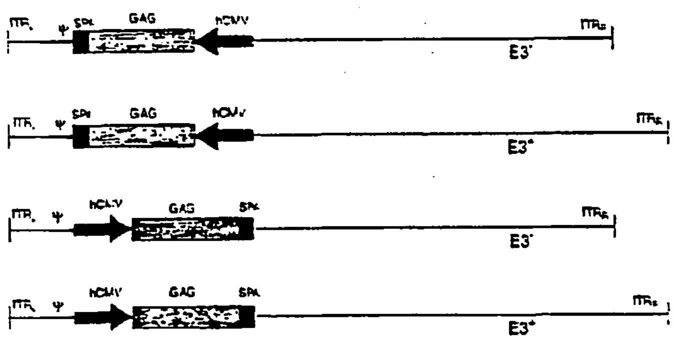


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

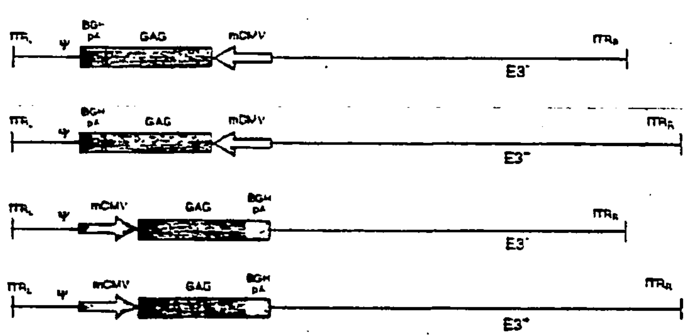


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

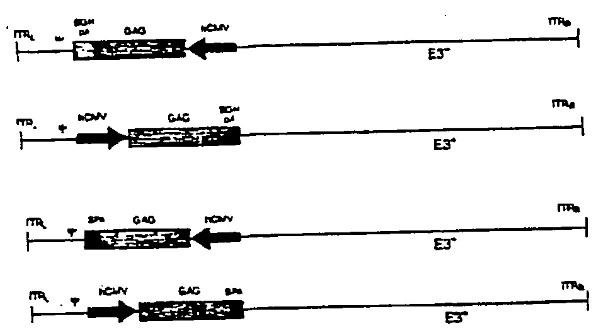


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

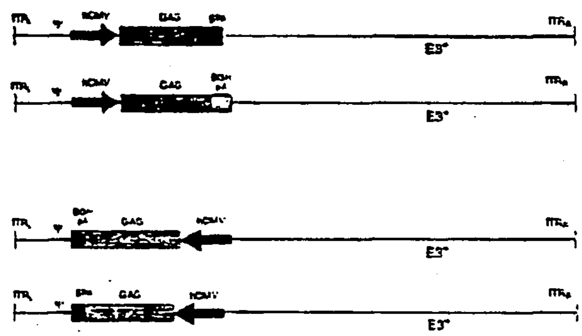


Figure 8B: Effect of polyadenylation signal

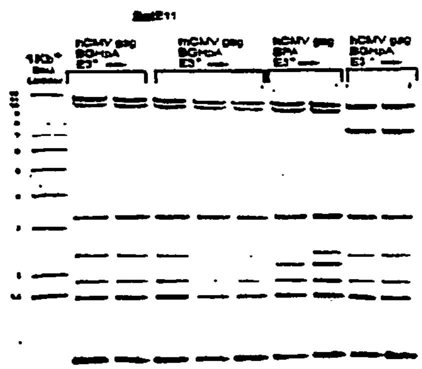


Figure 9: Viral DNA from the four Adgag candidates at P5, following *BstE11* digestion.

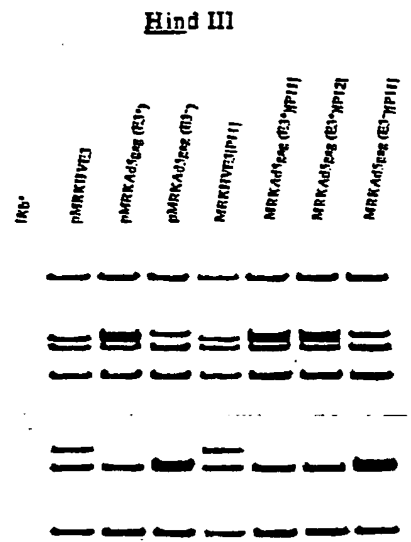


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

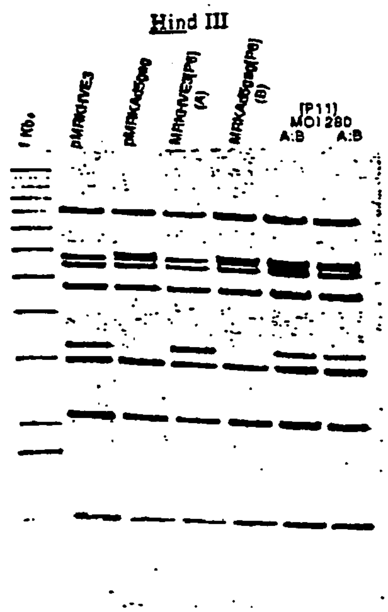


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

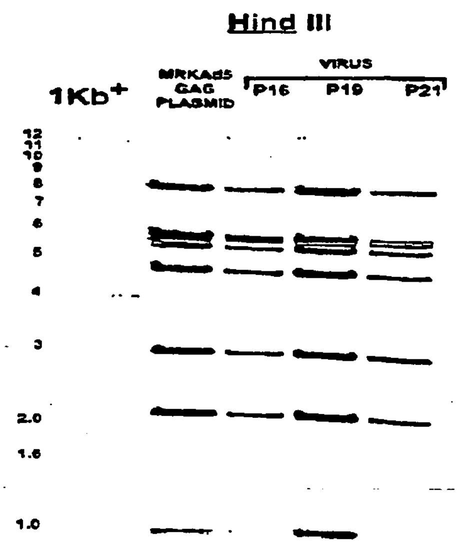
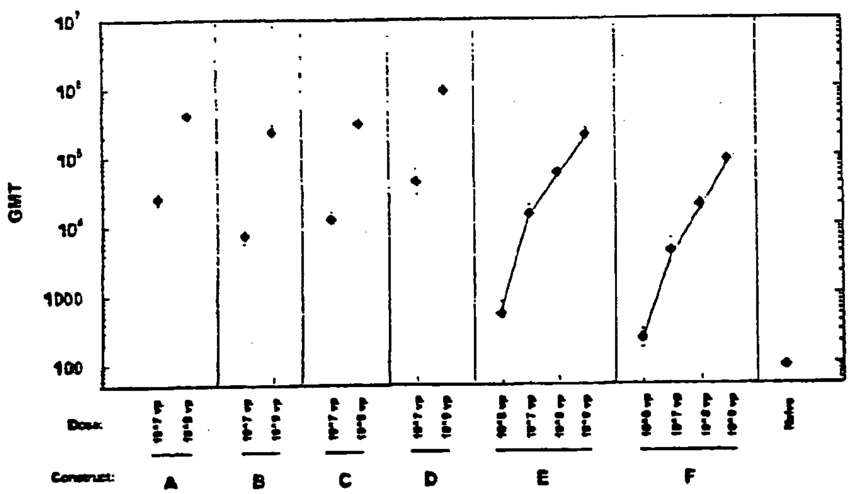


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁻ bCMV-FLgag-bGHpA; (C) MRKAd5 E3⁻ bCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



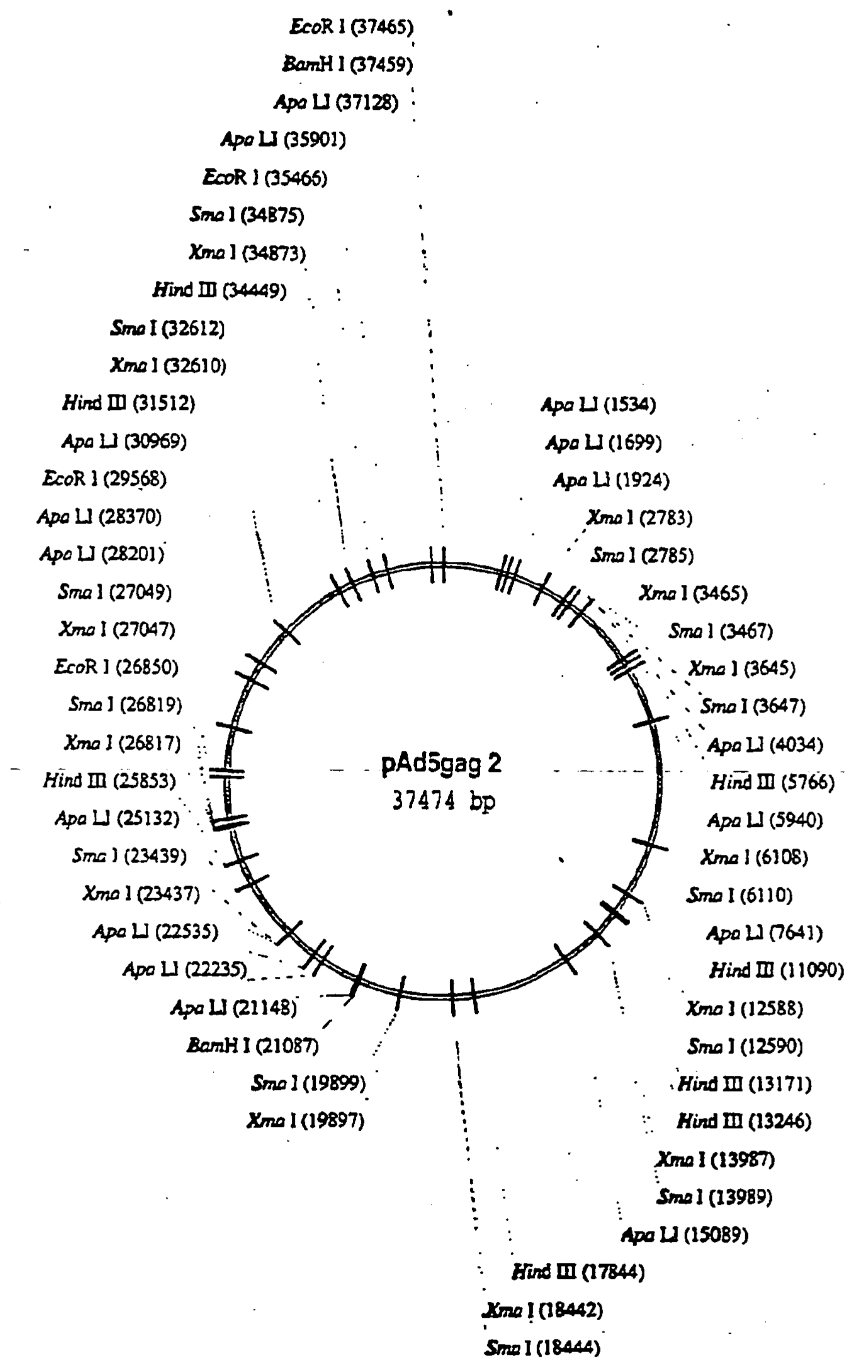

EcoR I (37465)
BamH I (37459)
Apa LI (37128)
Apa LI (35901)
EcoR I (35466)
Sma I (34875)
Xma I (34873)
Hind III (34449)
Sma I (32612)
Xma I (32610)
Hind III (31512)
Apa LI (30969)
EcoR I (29568)
Apa LI (28370)
Apa LI (28201)
Sma I (27049)
Xma I (27047)
EcoR I (26850)
Sma I (26819)
Xma I (26817)
Hind III (25853)
Apa LI (25132)
Sma I (23439)
Xma I (23437)
Apa LI (22535)
Apa LI (22235)
Apa LI (21148)
BamH I (21087)
Sma I (19899)
Xma I (19897)
Apa LI (1534)
Apa LI (1699)
Apa LI (1924)
Xma I (2783)
Sma I (2785)
Xma I (3465)
Sma I (3467)
Xma I (3645)
Sma I (3647)
Apa LI (4034)
Hind III (5766)
Apa LI (5940)
Xma I (6108)
Sma I (6110)
Apa LI (7641)
Hind III (11090)
Xma I (12588)
Sma I (12590)
Hind III (13171)
Hind III (13246)
Xma I (13987)
Sma I (13989)
Apa LI (15089)
Hind III (17844)
Xma I (18442)
Sma I (18444)
pAd5gag 2
37474 bp

Figure 14

pMRKAd5gag ME6B2

```
       PacI
1      TTCTTAATTA ACATCATCAA TAATATACCT TATTTTGGAT TGAAGCCAAT ATGATAATGA GGGGGTGGAG TTTGTGACGT GGCGCGGGGC GTGGGAACGG
       AAGAATTAAT TGTAGTAGTT ATTATATGGA ATAAAACCTA ACTTCGGTTA TACTATTACT CCCCCACCTC AAACACTGCA CCGCGCCCCG CACCCTTGCC
101    GGCGGGTGAC GTAGTAGTGT GGCGGAAGTG TGATGTTGCA AGTGTGGCGG AACACATGTA AGCGACGGAT GTGGCAAAAG TGACGTTTTT GGTGTGCGCC
       CCGCCCACTG CATCATCACA CCGCCTTCAC ACTACAACGT TCACACCGCC TTGTGTACAT TCGCTGCCTA CACCGTTTTC ACTGCAAAAA CCACACGCGG
201    GGTGTACACA GGAAGTGACA ATTTTCGCGC GGTTTTAGGC GGATGTTGTA GTAAATTTGG GCGTAACCGA GTAAGATTTG GCCATTTTCG CGGGAAAACT
       CCACATGTGT CCTTCACTGT TAAAAGCGCG CCAAAATCCG CCTACAACAT CATTTAAACC CGCATTGGCT CATTCTAAAC CGGTAAAAGC GCCCTTTTGA
301    GAATAAGAGG AAGTGAAATC TGAATAATTT TGTGTTACTC ATAGCGCGTA ATATTTGTCT AGGGCCGCGG GGACTTTGAC CGTTTACGTG GAGACTCGCC
       CTTATTCTCC TTCACTTTAG ACTTATTAAA ACACAATGAG TATCGCGCAT TATAAACAGA TCCCGGCGCC CCTGAAACTG GCAAATGCAC CTCTGAGCGG
401    CAGGTGTTTT TCTCAGGTGT TTTCCGCGTT CCGGGTCAAA GTTGGCGTTT TATTATTATA GGCGGCCGCG ATCCATTGCA TACGTTGTAT CCATATCATA
       GTCCACAAAA AGAGTCCACA AAAGGCGCAA GGCCCAGTTT CAACCGCAAA ATAATAATAT CCGCCGGCGC TAGGTAACGT ATGCAACATA GGTATAGTAT
501    ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA
       TATACATGTA AATATAACCG AGTACAGGTT GTAATGGCGG TACAACTGTA ACTAATAACT GATCAATAAT TATCATTAGT TAATGCCCCA GTAATCAAGT
601    TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT
       ATCGGGTATA TACCTCAAGG CGCAATGTAT TGAATGCCAT TTACCGGGCG GACCGACTGG CGGGTTGCTG GGGGCGGGTA ACTGCAGTTA TTACTGCATA
701    GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC
       CAAGGGTATC ATTGCGGTTA TCCCTGAAAG GTAACTGCAG TTACCCACCT CATAAATGCC ATTTGACGGG TGAACCGTCA TGTAGTTCAC ATAGTATACG
801    CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA
       GTTCATGCGG GGGATAACTG CAGTTACTGC CATTTACCGG GCGGACCGTA ATACGGGTCA TGTACTGGAA TACCCTGAAA GGATGAACCG TCATGTAGAT
901    CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC
       GCATAATCAG TAGCGATAAT GGTACCACTA CGCCAAAACC GTCATGTAGT TACCCGCACC TATCGCCAAA CTGAGTGCCC CTAAAGGTTC AGAGGTGGGG
1001   ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG
       TAACTGCAGT TACCCTCAAA CAAAACCGTG GTTTTAGTTG CCCTGAAAGG TTTTACAGCA TTGTTGAGGC GGGGTAACTG CGTTTACCCG CCATCCGCAC
1101   TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA
       ATGCCACCCT CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG CGGACCTCTG CGGTAGGTGC GACAAAACTG GAGGTATCTT CTGTGGCCCT
                                                                        BglII
1201   CCGATCCAGC CTCCGCGGCC GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGAGATCTAC CATGGGTGCT AGGGCTTCTG TGCTGTCTGG
       GGCTAGGTCG GAGGCGCCGG CCCTTGCCAC GTAACCTTGC GCCTAAGGGG CACGGTTCTC ACTCTAGATG GTACCCACGA TCCCGAAGAC ACGACAGACC
1301   TGGTGAGCTG GACAAGTGGG AGAAGATCAG GCTGAGGCCT GGTGGCAAGA AGAAGTACAA GCTAAAGCAC ATTGTGTGGG CCTCCAGGGA GCTGGAGAGG
       ACCACTCGAC CTGTTCACCC TCTTCTAGTC CGACTCCGGA CCACCGTTCT TCTTCATGTT CGATTTCGTG TAACACACCC GGAGGTCCCT CGACCTCTCC
1401   TTTGCTGTGA ACCCTGGCCT GCTGGAGACC TCTGAGGGGT GCAGGCAGAT CCTGGGCCAG CTCCAGCCCT CCCTGCAAAC AGGCTCTGAG GAGCTGAGGT
       AAACGACACT TGGGACCGGA CGACCTCTGG AGACTCCCCA CGTCCGTCTA GGACCCGGTC GAGGTCGGGA GGGACGTTTG TCCGAGACTC CTCGACTCCA
1501   CCCTGTACAA CACAGTGGCT ACCCTGTACT GTGTGCACCA GAAGATTGAT GTGAAGGACA CCAAGGAGGC CCTGGAGAAG ATTGAGGAGG AGCAGAACAA
       GGGACATGTT GTGTCACCGA TGGGACATGA CACACGTGGT CTTCTAACTA CACTTCCTGT GGTTCCTCCG GGACCTCTTC TAACTCCTCC TCGTCTTGTT
1601   GTCCAAGAAG AAGGCCCAGC AGGCTGCTGC TGCCACAGGC AACTCCAGCC AGGTGTCCCA GAACTACCCC ATTGTGCAGA ACCTCCAGGG CCAGATGGTG
       CAGGTTCTTC TTCCGGGTCG TCCGACGACG ACGGTGTCCG TTGAGGTCGG TCCACAGGGT CTTGATGGGG TAACACGTCT TGGAGGTCCC GGTCTACCAC
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Figure 15A

pMRKAd5gag MER682

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1701 CACCAGGCCA TCTCCCCCCG GACCCTGAAT GCCTGGGTGA AGGTGGTGGA GGAGAAGGCC TTCTCCCCTG AGGTGATCCC CATGTTCTCT GCCCTGTCTG
     GTGGTCCGGT AGAGGGGGGC CTGGGACTTA CGGACCCACT TCCACCACCT CCTCTTCCGG AAGAGGGGAC TCCACTAGGG GTACAAGAGA CGGGACAGAC
1801 AGGGTGCCAC CCCCCAGGAC CTGAACACCA TGCTGAACAC AGTGGGGGGC CATCAGGCTG CCATGCAGAT GCTGAAGGAG ACCATCAATG AGGAGGCTGC
     TCCCACGGTG GGGGGTCCTG GACTTGTGGT ACGACTTGTG TCACCCCCCG GTAGTCCGAC GGTACGTCTA CGACTTCCTC TGGTAGTTAC TCCTCCGACG
1901 TGAGTGGGAC AGGCTGCATC CTGTGCACGC TGGCCCCATT GCCCCCGGCC AGATGAGGGA GCCCAGGGGC TCTGACATTG CTGGCACCAC CTCCACCCTG
     ACTCACCCTG TCCGACGTAG GACACGTGCG ACCGGGGTAA CGGGGGCCGG TCTACTCCCT CGGGTCCCCG AGACTGTAAC GACCGTGGTG GAGGTGGGAC
2001 CAGGAGCAGA TTGGCTGGAT GACCAACAAC CCCCCCATCC CTGTGGGGGA AATCTACAAG AGGTGGATCA TCCTGGGCCT GAACAAGATT GTGAGGATGT
     GTCCTCGTCT AACCGACCTA CTGGTTGTTG GGGGGGTAGG GACACCCCCT TTAGATGTTC TCCACCTAGT AGGACCCGGA CTTGTTCTAA CACTCCTACA
2101 ACTCCCCCAC CTCCATCCTG GACATCAGGC AGGGCCCCAA GGAGCCCTTC AGGGACTATG TGGACAGGTT CTACAAGACC CTGAGGGCTG AGCAGGCCTC
     TGAGGGGGTG GAGGTAGGAC CTGTAGTCCG TCCCGGGGTT CCTCGGGAAG TCCCTGATAC ACCTGTCCAA GATGTTCTGG GACTCCCGAC TCGTCCGGAG
2201 CCAGGAGGTG AAGAACTGGA TGACAGAGAC CCTGCTGGTG CAGAATGCCA ACCCTGACTG CAAGACCATC CTGAAGGCCC TGGGCCCTGC TGCCACCCTG
     GGTCCTCCAC TTCTTGACCT ACTGTCTCTG GGACGACCAC GTCTTACGGT TGGGACTGAC GTTCTGGTAG GACTTCCGGG ACCCGGGACG ACGGTGGGAC
2301 GAGGAGATGA TGACAGCCTG CCAGGGGGTG GGGGGCCCTG GTCACAAGGC CAGGGTGCTG GCTGAGGCCA TGTCCCAGGT GACCAACTCC GCCACCATCA
     CTCCTCTACT ACTGTCGGAC GGTCCCCCAC CCCCCGGGAC CAGTGTTCCG GTCCCACGAC CGACTCCGGT ACAGGGTCCA CTGGTTGAGG CGGTGGTAGT
2401 TGATGCAGAG GGGCAACTTC AGGAACCAGA GGAAGACAGT GAAGTGCTTC AACTGTGGCA AGGTGGGCCA CATTGCCAAG AACTGTAGGG CCCCCAGGAA
     ACTACGTCTC CCCGTTGAAG TCCTTGGTCT CCTTCTGTCA CTTCACGAAG TTGACACCGT TCCACCCGGT GTAACGGTTC TTGACATCCC GGGGGTCCTT
2501 GAAGGGCTGC TGGAAGTGTG GCAAGGAGGG CCACCAGATG AAGGACTGCA ATGAGAGGCA GGCCAACTTC CTGGGCAAAA TCTGGCCCTC CCACAAGGGC
     CTTCCCGACG ACCTTCACAC CGTTCCTCCC GGTGGTCTAC TTCCTGACGT TACTCTCCGT CCGGTTGAAG GACCCGTTTT AGACCGGGAG GGTGTTCCCG
2601 AGGCCTGGCA ACTTCCTCCA GTCCAGGCCT GAGCCCACAG CCCCTCCCGA GGAGTCCTTC AGGTTTGGGG AGGAGAAGAC CACCCCCAGC CAGAAGCAGG
     TCCGGACCGT TGAAGGAGGT CAGGTCCGGA CTCGGGTGTC GGGGAGGGCT CCTCAGGAAG TCCAAACCCC TCCTCTTCTG GTGGGGGTCG GTCTTCGTCC
                                                                                                         BglII
2701 AGCCCATTGA CAAGGAGCTG TACCCCCTGG CCTCCCTGAG GTCCCTGTTT GGCAACGACC CCTCCTCCCA GTAAAATAAA GCCCGGGCAG ATCTGCTGTC
     TCGGGTAACT GTTCCTCGAC ATGGGGGACC GGAGGGACTC CAGGGACAAA CCGTTGCTGG GGAGGAGGGT CATTTTATTT CGGGCCCGTC TAGACGACAG
2801 CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA
     GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC ACGGAAGGAA CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT TTACTCCTTT
                                                                                                          SphI
2901 TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG
     AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC CCCACCCCAC CCCGTCCTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT CCGTACGACC
                           PvuI
                                AscI
3001 GGATGCGGTG GGCTCTATGG CCGATCGGCG CGCCGTACTG AAATGTGTGG GCGTGGCTTA AGGGTGGGAA AGAATATATA AGGTGGGGGT CTTATGTAGT
     CCTACGCCAC CCGAGATACC GGCTAGCCGC GCGGCATGAC TTTACACACC CGCACCGAAT TCCCACCCTT TCTTATATAT TCCACCCCCA GAATACATCA
                                                                                               SphI
3101 TTTGTATCTG TTTTGCAGCA GCCGCCGCCG CCATGAGCAC CAACTCGTTT GATGGAAGCA TTGTGAGCTC ATATTTGACA ACGCGCATGC CCCCATGGGC
     AAACATAGAC AAAACGTCGT CGGCGGCGGC GGTACTCGTG GTTGAGCAAA CTACCTTCGT AACACTCGAG TATAAACTGT TGCGCGTACG GGGGTACCCG
3201 CGGGGTGCGT CAGAATGTGA TGGGCTCCAG CATTGATGGT CGCCCCGTCC TGCCCGCAAA CTCTACTACC TTGACCTACG AGACCGTGTC TGGAACGCCG
     GCCCCACGCA GTCTTACACT ACCCGAGGTC GTAACTACCA GCGGGGCAGG ACGGGCGTTT GAGATGATGG AACTGGATGC TCTGGCACAG ACCTTGCGGC
```

Figure 15B

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PstI PstI
3301 TTGGAGACTG CAGCCTCCGC CGCCGCTTCA GCCGCTGCAG CCACCGCCCG CGGGATTGTG ACTGACTTTG CTTTCCTGAG CCCGCTTGCA AACAGTGCAG
AACCTCTGAC GTCGGAGGCG GCGGCGAAGT CGGCGACGTC GGTGGCGGGC GCCCTAACAC TGACTGAAAC GAAAGGACTC GGGCGAACGT TTGTCACGTC
3401 CTTCCCGTTC ATCCGCCCGC GATGACAAGT TGACGGCTCT TTTGGCACAA TTGGATTCTT TGACCCGGGA ACTTAATGTC GTTTCTCAGC AGCTGTTGGA
GAAGGGCAAG TAGGCGGGCG CTACTGTTCA ACTGCCGAGA AAACCGTGTT AACCTAAGAA ACTGGGCCCT TGAATTACAG CAAAGAGTCG TCGACAACCT
3501 TCTGCGCCAG CAGGTTTCTG CCCTGAAGGC TTCCTCCCCT CCCAATGCGG TTTAAAACAT AAATAAAAAA CCAGACTCTG TTTGGATTTG GATCAAGCAA
AGACGCGGTC GTCCAAAGAC GGGACTTCCG AAGGAGGGGA GGGTTACGCC AAATTTTGTA TTTATTTTTT GGTCTGAGAC AAACCTAAAC CTAGTTCGTT
3601 GTGTCTTGCT GTCTTTATTT AGGGGTTTTG CGCGCGCGGT AGGCCCGGGA CCAGCGGTCT CGGTCGTTGA GGGTCCTGTG TATTTTTTCC AGGACGTGGT
CACAGAACGA CAGAAATAAA TCCCCAAAAC GCGCGCGCCA TCCGGGCCCT GGTCGCCAGA GCCAGCAACT CCCAGGACAC ATAAAAAAGG TCCTGCACCA

PstI
3701 AAAGGTGACT CTGGATGTTC AGATACATGG GCATAAGCCC GTCTCTGGGG TGGAGGTAGC ACCACTGCAG AGCTTCATGC TGCGGGGTGG TGTTGTAGAT
TTTCCACTGA GACCTACAAG TCTATGTACC CGTATTCGGG CAGAGACCCC ACCTCCATCG TGGTGACGTC TCGAAGTACG ACGCCCCACC ACAACATCTA
3801 GATCCAGTCG TAGCAGGAGC GCTGGGCGTG GTGCCTAAAA ATGTCTTTCA GTAGCAAGCT GATTGCCAGG GGCAGGCCCT TGGTGTAAGT GTTTACAAAG
CTAGGTCAGC ATCGTCCTCG CGACCCGCAC CACGGATTTT TACAGAAAGT CATCGTTCGA CTAACGGTCC CCGTCCGGGA ACCACATTCA CAAATGTTTC
3901 CGGTTAAGCT GGGATGGGTG CATACGTGGG GATATGAGAT GCATCTTGGA CTGTATTTTT AGGTTGGCTA TGTTCCCAGC CATATCCCTC CGGGGATTCA
GCCAATTCGA CCCTACCCAC GTATGCACCC CTATACTCTA CGTAGAACCT GACATAAAAA TCCAACCGAT ACAAGGGTCG GTATAGGGAG GCCCCTAAGT
4001 TGTTGTGCAG AACCACCAGC ACAGTGTATC CGGTGCACTT GGGAAATTTG TCATGTAGCT TAGAAGGAAA TGCGTGGAAG AACTTGGAGA CGCCCTTGTG
ACAACACGTC TTGGTGGTCG TGTCACATAG GCCACGTGAA CCCTTTAAAC AGTACATCGA ATCTTCCTTT ACGCACCTTC TTGAACCTCT GCGGGAACAC
4101 ACCTCCAAGA TTTTCCATGC ATTCGTCCAT AATGATGGCA ATGGGCCCAC GGGCGGCGGC CTGGGCGAAG ATATTTCTGG GATCACTAAC GTCATAGTTG
TGGAGGTTCT AAAAGGTACG TAAGCAGGTA TTACTACCGT TACCCGGGTG CCCGCCGCCG GACCCGCTTC TATAAAGACC CTAGTGATTG CAGTATCAAC
4201 TGTTCCAGGA TGAGATCGTC ATAGGCCATT TTTACAAAGC GCGGGCGGAG GGTGCCAGAC TGCGGTATAA TGGTTCCATC CGGCCCAGGG GCGTAGTTA
ACAAGGTCCT ACTCTAGCAG TATCCGGTAA AAATGTTTCG CGCCCGCCTC CCACGGTCTG ACGCCATATT ACCAAGGTAG GCCGGGTCCC CGCATCAAT
4301 CCTCACAGAT TTGCATTTCC CACGCTTTGA GTTCAGATGG GGGGATCATG TCTACCTGCG GGGCGATGAA GAAAACGGTT TCCGGGGTAG GGGAGATCAG
GGAGTGTCTA AACGTAAAGG GTGCGAAACT CAAGTCTACC CCCCTAGTAC AGATGGACGC CCCGCTACTT CTTTTGCCAA AGGCCCCATC CCCTCTAGTC
PstI

4401 CTGGGAAGAA AGCAGGTTCC TGAGCAGCTG CGACTTACCG CAGCCGGTGG GCCCGTAAAT CACACCTATT ACCGGCTGCA ACTGGTAGTT AAGAGAGCTG
GACCCTTCTT TCGTCCAAGG ACTCGTCGAC GCTGAATGGC GTCGGCCACC CGGGCATTTA GTGTGGATAA TGGCCGACGT TGACCATCAA TTCTCTCGAC
PstI
4501 CAGCTGCCGT CATCCCTGAG CAGGGGGGCC ACTTCGTTAA GCATGTCCCT GACTCGCATG TTTTCCCTGA CCAAATCCGC CAGAAGGCGC TCGCCGCCCA
GTCGACGGCA GTAGGGACTC GTCCCCCCGG TGAAGCAATT CGTACAGGGA CTGAGCGTAC AAAAGGGACT GGTTTAGGCG GTCTTCCGCG AGCGGCGGGT
SphI

4601 GCGATAGCAG TTCTTGCAAG GAAGCAAAGT TTTTCAACGG TTTGAGACCG TCCGCCGTAG GCATGCTTTT GAGCGTTTGA CCAAGCAGTT CCAGGCGGTC
CGCTATCGTC AAGAACGTTC CTTCGTTTCA AAAAGTTGCC AAACTCTGGC AGGCGGCATC CGTACGAAAA CTCGCAAACT GGTTCGTCAA GGTCCGCCAG
4701 CCACAGCTCG GTCACCTGCT CTACGGCATC TCGATCCAGC ATATCTCCTC GTTTCGCGGG TTGGGGCGGC TTTCGCTGTA CGGCAGTAGT CGGTGCTCGT
GGTGTCGAGC CAGTGGACGA GATGCCGTAG AGCTAGGTCG TATAGAGGAG CAAAGCGCCC AACCCCGCCG AAAGCGACAT GCCGTCATCA GCCACGAGCA
4801 CCAGACGGGC CAGGGTCATG TCTTTCCACG GGCGCAGGGT CCTCGTCAGC GTAGTCTGGG TCACGGTGAA GGGGTGCGCT CCGGGCTGCG CGCTGGCCAG
GGTCTGCCCG GTCCCAGTAC AGAAAGGTGC CCGCGTCCCA GGAGCAGTCG CATCAGACCC AGTGCCACTT CCCCACGCGA GGCCCGACGC GCGACCGGTC

Figure 15C

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4901  GGTGCGCTTG AGGCTGGTCC TGCTGGTGCT GAAGCGCTGC CGGTCTTCGC CCTGCGCGTC GGCCAGGTAG CATTTGACCA TGGTGTCATA GTCCAGCCCC
      CCACGCGAAC TCCGACCAGG ACGACCACGA CTTCGCGACG GCCAGAAGCG GGACGCGCAG CCGGTCCATC GTAAACTGGT ACCACAGTAT CAGGTCGGGG
5001  TCCGCGGCGT GGCCCTTGGC GCGCAGCTTG CCCTTGGAGG AGGCGCCGCA CGAGGGGCAG TGCAGACTTT TGAGGGCGTA GAGCTTGGGC GCGAGAAATA
      AGGCGCCGCA CCGGGAACCG CGCGTCGAAC GGGAACCTCC TCCGCGGCGT GCTCCCCGTC ACGTCTGAAA ACTCCCGCAT CTCGAACCCG CGCTCTTTAT
5101  CCGATTCCGG GGAGTAGGCA TCCGCGCCGC AGGCCCCGCA GACGGTCTCG CATTCCACGA GCCAGGTGAG CTCTGGCCGT TCGGGGTCAA AAACCAGGTT
      GGCTAAGGCC CCTCATCCGT AGGCGCGGCG TCCGGGGCGT CTGCCAGAGC GTAAGGTGCT CGGTCCACTC GAGACCGGCA AGCCCCAGTT TTTGGTCCAA
                                                                                                 Bsu1107I
5201  TCCCCCATGC TTTTTGATGC GTTTCTTACC TCTGGTTTCC ATGAGCCGGT GTCCACGCTC GGTGACGAAA AGGCTGTCCG TGTCCCCGTA TACAGACTTG
      AGGGGGTACG AAAAACTACG CAAAGAATGG AGACCAAAGG TACTCGGCCA CAGGTGCGAG CCACTGCTTT TCCGACAGGC ACAGGGGCAT ATGTCTGAAC
                 XhoI
5301  AGAGGCCTGT CCTCGAGCGG TGTTCCGCGG TCCTCCTCGT ATAGAAACTC GGACCACTCT GAGACAAAGG CTCGCGTCCA GGCCAGCACG AAGGAGGCTA
      TCTCCGGACA GGAGCTCGCC ACAAGGCGCC AGGAGGAGCA TATCTTTGAG CCTGGTGAGA CTCTGTTTCC GAGCGCAGGT CCGGTCGTGC TTCCTCCGAT
5401  AGTGGGAGGG GTAGCGGTCG TTGTCCACTA GGGGGTCCAC TCGCTCCAGG GTGTGAAGAC ACATGTCGCC CTCTTCGGCA TCAAGGAAGG TGATTGGTTT
      TCACCCTCCC CATCGCCAGC AACAGGTGAT CCCCCAGGTG AGCGAGGTCC CACACTTCTG TGTACAGCGG GAGAAGCCGT AGTTCCTTCC ACTAACCAAA
5501  GTAGGTGTAG GCCACGTGAC CGGGTGTTCC TGAAGGGGGG CTATAAAAGG GGGTGGGGGC GCGTTCGTCC TCACTCTCTT CCGCATCGCT GTCTGCGAGG
      CATCCACATC CGGTGCACTG GCCCACAAGG ACTTCCCCCC GATATTTTCC CCCACCCCCG CGCAAGCAGG AGTGAGAGAA GGCGTAGCGA CAGACGCTCC
5601  GCCAGCTGTT GGGGTGAGTA CTCCCTCTGA AAAGCGGGCA TGACTTCTGC GCTAAGATTG TCAGTTTCCA AAAACGAGGA GGATTTGATA TTCACCTGGC
      CGGTCGACAA CCCCACTCAT GAGGGAGACT TTTCGCCCGT ACTGAAGACG CGATTCTAAC AGTCAAAGGT TTTTGCTCCT CCTAAACTAT AAGTGGACCG
                                                                               HindIII
5701  CCGCGGTGAT GCCTTTGAGG GTGGCCGCAT CCATCTGGTC AGAAAAGACA ATCTTTTTGT TGTCAAGCTT GGTGGCAAAC GACCCGTAGA GGGCGTTGGA
      GGCGCCACTA CGGAAACTCC CACCGGCGTA GGTAGACCAG TCTTTTCTGT TAGAAAAACA ACAGTTCGAA CCACCGTTTG CTGGGCATCT CCCGCAACCT
                                                   PvuI
5801  CAGCAACTTG GCGATGGAGC GCAGGGTTTG GTTTTTGTCG CGATCGGCGC GCTCCTTGGC CGCGATGTTT AGCTGCACGT ATTCGCGCGC AACGCACCGC
      GTCGTTGAAC CGCTACCTCG CGTCCCAAAC CAAAAACAGC GCTAGCCGCG CGAGGAACCG GCGCTACAAA TCGACGTGCA TAAGCGCGCG TTGCGTGGCG
5901  CATTCGGGAA AGACGGTGGT GCGCTCGTCG GGCACCAGGT GCACGCGCCA ACCGCGGTTG TGCAGGGTGA CAAGGTCAAC GCTGGTGGCT ACCTCTCCGC
      GTAAGCCCTT TCTGCCACCA CGCGAGCAGC CCGTGGTCCA CGTGCGCGGT TGGCGCCAAC ACGTCCCACT GTTCCAGTTG CGACCACCGA TGGAGAGGCG
6001  GTAGGCGCTC GTTGGTCCAG CAGAGGCGGC CGCCCTTGCG CGAGCAGAAT GGCGGTAGGG GGTCTAGCTG CGTCTCGTCC GGGGGGTCTG CGTCCACGGT
      CATCCGCGAG CAACCAGGTC GTCTCCGCCG GCGGGAACGC GCTCGTCTTA CCGCCATCCC CCAGATCGAC GCAGAGCAGG CCCCCCAGAC GCAGGTGCCA
6101  AAAGACCCCG GGCAGCAGGC GCGCGTCGAA GTAGTCTATC TTGCATCCTT GCAAGTCTAG CGCCTGCTGC CATGCGCGGG CGGCAAGCGC GCGCTCGTAT
      TTTCTGGGGC CCGTCGTCCG CGCGCAGCTT CATCAGATAG AACGTAGGAA CGTTCAGATC GCGGACGACG GTACGCGCCC GCCGTTCGCG CGCGAGCATA
6201  GGGTTGAGTG GGGGACCCCA TGGCATGGGG TGGGTGAGCG CGGAGGCGTA CATGCCGCAA ATGTCGTAAA CGTAGAGGGG CTCTCTGAGT ATTCCAAGAT
      CCCAACTCAC CCCCTGGGGT ACCGTACCCC ACCCACTCGC GCCTCCGCAT GTACGGCGTT TACAGCATTT GCATCTCCCC GAGAGACTCA TAAGGTTCTA
6301  ATGTAGGGTA GCATCTTCCA CCGCGGATGC TGGCGCGCAC GTAATCGTAT AGTTCGTGCG AGGGAGCGAG GAGGTCGGGA CCGAGGTTGC TACGGGCGGG
      TACATCCCAT CGTAGAAGGT GGCGCCTACG ACCGCGCGTG CATTAGCATA TCAAGCACGC TCCCTCGCTC CTCCAGCCCT GGCTCCAACG ATGCCCGCCC
6401  CTGCTCTGCT CGGAAGACTA TCTGCCTGAA GATGGCATGT GAGTTGGATG ATATGGTTGG ACGCTGGAAG ACGTTGAAGC TGGCGTCTGT GAGACCTACC
      GACGAGACGA GCCTTCTGAT AGACGGACTT CTACCGTACA CTCAACCTAC TATACCAACC TGCGACCTTC TGCAACTTCG ACCGCAGACA CTCTGGATGG
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Figure 15D

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6501  GCGTCACGCA CGAAGGAGGC GTAGGAGTCG CGCAGCTTGT TGACCAGCTC GCGGGTGACC TGCACGTCTA GGGCGCAGTA GTCCAGGGTT TCCTTGATGA
      CGCAGTGCGT GCTTCCTCCG CATCCTCAGC GCGTCGAACA ACTGGTCGAG CGCCCACTGG ACGTGCAGAT CCCGCGTCAT CAGGTCCCAA AGGAACTACT
6601  TGTCATACTT ATCCTGTCCC TTTTTTTTCC ACAGCTCGCG GTTGAGGACA AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC
      ACAGTATGAA TAGGACAGGG AAAAAAAAGG TGTCGAGCGC CAACTCCTGT TTGAGAAGCG CCAGAAAGGT CATGAGAACC TAGCCTTTGG GCAGCCGGAG
6701  CGAACGGTAA GAGCCTAGCA TGTAGAACTG GTTGACGGCC TGGTAGGCGC AGCATCCCTT TTCTACGGGT AGCGCGTATG CCTGCGCGGC CTTCCGGAGC
      GCTTGCCATT CTCGGATCGT ACATCTTGAC CAACTGCCGG ACCATCCGCG TCGTAGGGAA AAGATGCCCA TCGCGCATAC GGACGCGCCG GAAGGCCTCG
6801  GAGGTGTGGG TGAGCGCAAA GGTGTCCCTG ACCATGACTT TGAGGTACTG GTATTTGAAG TCAGTGTCGT CGCATCCGCC CTGCTCCCAG AGCAAAAAGT
      CTCCACACCC ACTCGCGTTT CCACAGGGAC TGGTACTGAA ACTCCATGAC CATAAACTTC AGTCACAGCA GCGTAGGCGG GACGAGGGTC TCGTTTTTCA
6901  CCGTGCGCTT TTTGGAACGC GGATTTGGCA GGGCGAAGGT GACATCGTTG AAGAGTATCT TTCCCGCGCG AGGCATAAAG TTGCGTGTGA TGCGGAAGGG
      GGCACGCGAA AAACCTTGCG CCTAAACCGT CCCGCTTCCA CTGTAGCAAC TTCTCATAGA AAGGGCGCGC TCCGTATTTC AACGCACACT ACGCCTTCCC
7001  TCCCGGCACC TCGGAACGGT TGTTAATTAC CTGGGCGGCG AGCACGATCT CGTCAAAGCC GTTGATGTTG TGGCCCACAA TGTAAAGTTC CAAGAAGCGC
      AGGGCCGTGG AGCCTTGCCA ACAATTAATG GACCCGCCGC TCGTGCTAGA GCAGTTTCGG CAACTACAAC ACCGGGTGTT ACATTTCAAG GTTCTTCGCG
7101  GGGATGCCCT TGATGGAAGG CAATTTTTTA AGTTCCTCGT AGGTGAGCTC TTCAGGGGAG CTGAGCCCGT GCTCTGAAAG GGCCCAGTCT GCAAGATGAG
      CCCTACGGGA ACTACCTTCC GTTAAAAAAT TCAAGGAGCA TCCACTCGAG AAGTCCCCTC GACTCGGGCA CGAGACTTTC CCGGGTCAGA CGTTCTACTC
7201  GGTTGGAAGC GACGAATGAG CTCCACAGGT CACGGGCCAT TAGCATTTGC AGGTGGTCGC GAAAGGTCCT AAACTGGCGA CCTATGGCCA TTTTTTCTGG
      CCAACCTTCG CTGCTTACTC GAGGTGTCCA GTGCCCGGTA ATCGTAAACG TCCACCAGCG CTTTCCAGGA TTTGACCGCT GGATACCGGT AAAAAAGACC
7301  GGTGATGCAG TAGAAGGTAA GCGGGTCTTG TTCCCAGCGG TCCCATCCAA GGTTCGCGGC TAGGTCTCGC GCGGCAGTCA CTAGAGGCTC ATCTCCGCCG
      CCACTACGTC ATCTTCCATT CGCCCAGAAC AAGGGTCGCC AGGGTAGGTT CCAAGCGCCG ATCCAGAGCG CGCCGTCAGT GATCTCCGAG TAGAGGCGGC
7401  AACTTCATGA CCAGCATGAA GGGCACGAGC TGCTTCCCAA AGGCCCCCAT CCAAGTATAG GTCTCTACAT CGTAGGTGAC AAAGAGACGC TCGGTGCGAG
      TTGAAGTACT GGTCGTACTT CCCGTGCTCG ACGAAGGGTT TCCGGGGGTA GGTTCATATC CAGAGATGTA GCATCCACTG TTTCTCTGCG AGCCACGCTC
                  PvuI
7501  GATGCGAGCC GATCGGGAAG AACTGGATCT CCCGCCACCA ATTGGAGGAG TGGCTATTGA TGTGGTGAAA GTAGAAGTCC CTGCGACGGG CCGAACACTC
      CTACGCTCGG CTAGCCCTTC TTGACCTAGA GGGCGGTGGT TAACCTCCTC ACCGATAACT ACACCACTTT CATCTTCAGG GACGCTGCCC GGCTTGTGAG
7601  GTGCTGGCTT TTGTAAAAAC GTGCGCAGTA CTGGCAGCGG TGCACGGGCT GTACATCCTG CACGAGGTTG ACCTGACGAC CGCGCACAAG GAAGCAGAGT
      CACGACCGAA AACATTTTTG CACGCGTCAT GACCGTCGCC ACGTGCCCGA CATGTAGGAC GTGCTCCAAC TGGACTGCTG GCGCGTGTTC CTTCGTCTCA
                                                                                       XhoI
7701  GGGAATTTGA GCCCCTCGCC TGGCGGGTTT GGCTGGTGGT CTTCTACTTC GGCTGCTTGT CCTTGACCGT CTGGCTGCTC GAGGGGAGTT ACGGTGGATC
      CCCTTAAACT CGGGGAGCGG ACCGCCCAAA CCGACCACCA GAAGATGAAG CCGACGAACA GGAACTGGCA GACCGACGAG CTCCCCTCAA TGCCACCTAG
7801  GGACCACCAC GCCGCGCGAG CCCAAAGTCC AGATGTCCGC GCGCGGCGGT CGGAGCTTGA TGACAACATC GCGCAGATGG GAGCTGTCCA TGGTCTGGAG
      CCTGGTGGTG CGGCGCGCTC GGGTTTCAGG TCTACAGGCG CGCGCCGCCA GCCTCGAACT ACTGTTGTAG CGCGTCTACC CTCGACAGGT ACCAGACCTC
                                         PstI
7901  CTCCCGCGGC GTCAGGTCAG GCGGGAGCTC CTGCAGGTTT ACCTCGCATA GACGGGTCAG GGCGCGGGCT AGATCCAGGT GATACCTAAT TTCCAGGGGC
      GAGGGCGCCG CAGTCCAGTC CGCCCTCGAG GACGTCCAAA TGGAGCGTAT CTGCCCAGTC CCGCGCCCGA TCTAGGTCCA CTATGGATTA AAGGTCCCCG
                                                                    KpnI
8001  TGGTTGGTGG CGGCGTCGAT GGCTTGCAAG AGGCCGCATC CCCGCGGCGC GACTACGGTA CCGCGCGGCG GGCGGTGGGC CGCGGGGGTG TCCTTGGATG
      ACCAACCACC GCCGCAGCTA CCGAACGTTC TCCGGCGTAG GGGCGCCGCG CTGATGCCAT GGCGCGCCGC CCGCCACCCG GCGCCCCCAC AGGAACCTAC
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Figure 15E

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8101  ATGCATCTAA AAGCCGTGAC GCGGGCGAGC CCCCGGAGGT AGGGGGGGCT CCGGACCCGC CGGGAGAGGG GGCAGGGGCA CGTCGGCGCC GCGCGCGGGC
      TACGTAGATT TTCGCCACTG CGCCCGCTCG GGGGCCTCCA TCCCCCCCGA GGCCTGGGCG GCCCTCTCCC CCGTCCCCGT GCAGCCGCGG CGCGCGCCCG
8201  AGGAGCTGGT GCTGCGCGCG TAGGTTGCTG GCGAACGCGA CGACGGCGCG GTTGATCTCC TGAATCTGGC GCCTCTGCGT GAAGACGACG GGCCCGGTGA
      TCCTCGACCA CGACGCGCGC ATCCAACGAC CGCTTGCGCT GCTGCCGCGC CAACTAGAGG ACTTAGACCG CGGAGACGCA CTTCTGCTGC CCGGGCCACT
8301  GCTTGAACCT GAAAGAGAGT TCGACAGAAT CAATTTCGGT GTCGTTGACG GCGGCCTGGC GCAAAATCTC CTGCACGTCT CCTGAGTTGT CTTGATAGGC
      CGAACTTGGA CTTTCTCTCA AGCTGTCTTA GTTAAAGCCA CAGCAACTGC CGCCGGACCG CGTTTTAGAG GACGTGCAGA GGACTCAACA GAACTATCCG
                                              BglII
8401  GATCTCGGCC ATGAACTGCT CGATCTCTTC CTCCTGGAGA TCTCCGCGTC CGGCTCGCTC CACGGTGGCG GCGAGGTCGT TGGAAATGCG GGCCATGAGC
      CTAGAGCCGG TACTTGACGA GCTAGAGAAG GAGGACCTCT AGAGGCGCAG GCCGAGCGAG GTGCCACCGC CGCTCCAGCA ACCTTTACGC CCGGTACTCG
8501  TGCGAGAAGG CGTTGAGGCC TCCCTCGTTC CAGACGCGGC TGTAGACCAC GCCCCCTTCG GCATCGCGGG CGCGCATGAC CACCTGCGCG AGATTGAGCT
      ACGCTCTTCC GCAACTCCGG AGGGAGCAAG GTCTGCGCCG ACATCTGGTG CGGGGGAAGC CGTAGCGCCC GCGCGTACTG GTGGACGCGC TCTAACTCGA
8601  CCACGTGCCG GGCGAAGACG GCGTAGTTTC GCAGGCGCTG AAAGAGGTAG TTGAGGGTGG TGGCGGTGTG TTCTGCCACG AAGAAGTACA TAACCCAGCG
      GGTGCACGGC CCGCTTCTGC CGCATCAAAG CGTCCGCGAC TTTCTCCATC AACTCCCACC ACCGCCACAC AAGACGGTGC TTCTTCATGT ATTGGGTCGC
                  EcoRV
8701  TCGCAACGTG GATTCGTTGA TATCCCCCAA GGCCTCAAGG CGCTCCATGG CCTCGTAGAA GTCCACGGCG AAGTTGAAAA ACTGGGAGTT GCGCGCCGAC
      AGCGTTGCAC CTAAGCAACT ATAGGGGGTT CCGGAGTTCC GCGAGGTACC GGAGCATCTT CAGGTGCCGC TTCAACTTTT TGACCCTCAA CGCGCGGCTG
8801  ACGGTTAACT CCTCCTCCAG AAGACGGATG AGCTCGGCGA CAGTGTCGCG CACCTCGCGC TCAAAGGCTA CAGGGGCCTC TTCTTCTTCT TCAATCTCCT
      TGCCAATTGA GGAGGAGGTC TTCTGCCTAC TCGAGCCGCT GTCACAGCGC GTGGAGCGCG AGTTTCCGAT GTCCCCGGAG AAGAAGAAGA AGTTAGAGGA
                                                                                                    SalI
8901  CTTCCATAAG GGCCTCCCCT TCTTCTTCTT CTGGCGGCGG TGGGGGAGGG GGGACACGGC GGCGACGACG GCGCACCGGG AGGCGGTCGA CAAAGCGCTC
      GAAGGTATTC CCGGAGGGGA AGAAGAAGAA GACCGCCGCC ACCCCCTCCC CCCTGTGCCG CCGCTGCTGC CGCGTGGCCC TCCGCCAGCT GTTTCGCGAG
9001  GATCATCTCC CCGCGGCGAC GGCGCATGGT CTCGGTGACG GCGCGGCCGT TCTCGCGGGG GCGCAGTTGG AAGACGCCGC CCGTCATGTC CCGGTTATGG
      CTAGTAGAGG GGCGCCGCTG CCGCGTACCA GAGCCACTGC CGCGCCGGCA AGAGCGCCCC CGCGTCAACC TTCTGCGGCG GGCAGTACAG GGCCAATACC
9101  GTTGGCGGGG GGCTGCCATG CGGCAGGGAT ACGGCGCTAA CGATGCATCT CAACAATTGT TGTGTAGGTA CTCCGCCGCC GAGGGACCTG AGCGAGTCC
      CAACCGCCCC CCGACGGTAC GCCGTCCCTA TGCCGCGATT GCTACGTAGA GTTGTTAACA ACACATCCAT GAGGCGGCGG CTCCCTGGAC TCGCTCAGG
                     XhoI
9201  CATCGACCGG ATCGGAAAAC CTCTCGAGAA AGGCGTCTAA CCAGTCACAG TCGCAAGGTA GGCTGAGCAC CGTGGCGGGC GGCAGCGGGC GGCGGTCGGG
      GTAGCTGGCC TAGCCTTTTG GAGAGCTCTT TCCGCAGATT GGTCAGTGTC AGCGTTCCAT CCGACTCGTG GCACCGCCCG CCGTCGCCCG CCGCCAGCCC
                                                                          SalI
9301  GTTGTTTCTG GCGGAGGTGC TGCTGATGAT GTAATTAAAG TAGGCGGTCT TGAGACGGCG GATGGTCGAC AGAAGCACCA TGTCCTTGGG TCCGGCCTGC
      CAACAAAGAC CGCCTCCACG ACGACTACTA CATTAATTTC ATCCGCCAGA ACTCTGCCGC CTACCAGCTG TCTTCGTGGT ACAGGAACCC AGGCCGGACG
9401  TGAATGCGCA GGCGGTCGGC CATGCCCCAG GCTTCGTTTT GACATCGGCG CAGGTCTTTG TAGTAGTCTT GCATGAGCCT TTCTACCGGC ACTTCTTCT
      ACTTACGCGT CCGCCAGCCG GTACGGGGTC CGAAGCAAAA CTGTAGCCGC GTCCAGAAAC ATCATCAGAA CGTACTCGGA AAGATGGCCG TGAAGAAGA
9501  CTCCTTCCTC TTGTCCTGCA TCTCTTGCAT CTATCGCTGC GGCGGCGGCG GAGTTTGGCC GTAGGTGGCG CCCTCTTCCT CCCATGCGTG TGACCCCGA
      GAGGAAGGAG AACAGGACGT AGAGAACGTA GATAGCGACG CCGCCGCCGC CTCAAACCGG CATCCACCGC GGGAGAAGGA GGGTACGCAC ACTGGGGCT
9601  GCCCCTCATC GGCTGAAGCA GGGCTAGGTC GGCGACAACG CGCTCGGCTA ATATGGCCTG CTGCACCTGC GTGAGGGTAG ACTGGAAGTC ATCCATGTCC
      CGGGGAGTAG CCGACTTCGT CCCGATCCAG CCGCTGTTGC GCGAGCCGAT TATACCGGAC GACGTGGACG CACTCCCATC TGACCTTCAG TAGGTACAGG
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Figure 15F

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                             XhoI
9701   ACAAAGCGGT GGTATGCGCC CGTGTTGATG GTGTAAGTGC AGTTGGCCAT AACGGACCAG TTAACGGTCT GGTGACCCGG CTGCGAGAGC TCGGTGTACC
       TGTTTCGCCA CCATACGCGG GCACAACTAC CACATTCACG TCAACCGGTA TTGCCTGGTC AATTGCCAGA CCACTGGGCC GACGCTCTCG AGCCACATGG

9801   TGAGACGCGA GTAAGCCCTC GAGTCAAATA CGTAGTCGTT GCAAGTCCGC ACCAGGTACT GGTATCCCAC CAAAAAGTGC GGCGGCGGCT GGCGGTAGAG
       ACTCTGCGCT CATTCGGGAG CTCAGTTTAT GCATCAGCAA CGTTCAGGCG TGGTCCATGA CCATAGGGTG GTTTTTCACG CCGCCGCCGA CCGCCATCTC
                                       BglII                  EcoRV
9901   GGGCCAGCGT AGGGTGGCCG GGGCTCCGGG GGCGAGATCT TCCAACATAA GGCGATGATA TCCGTAGATG TACCTGGACA TCCAGGTGAT GCCGGCGGCG
       CCCGGTCGCA TCCCACCGGC CCCGAGGCCC CCGCTCTAGA AGGTTGTATT CCGCTACTAT AGGCATCTAC ATGGACCTGT AGGTCCACTA CGGCCGCCGC

10001  GTGGTGGAGG CGCGCGGAAA GTCGCGGACG CGGTTCCAGA TGTTGCGCAG CGGCAAAAAG TGCTCCATGG TCGGGACGCT CTGGCCGGTC AGGCGCGCCG
       CACCACCTCC GCGCGCCTTT CAGCGCCTGC GCCAAGGTCT ACAACGCGTC GCCGTTTTTC ACGAGGTACC AGCCCTGCGA GACCGGCCAG TCCGCGCGGC
                  XbaI
10101  AATCGTTGAC GCTCTAGACC GTGCAAAAGG AGAGCCTGTA AGCGGGCACT CTTCCGTGGT CTGGTGGATA AATTCGCAAG GGTATCATGG CGGACGACCG
       TTAGCAACTG CGAGATCTGG CACGTTTTCC TCTCGGACAT TCGCCCGTGA GAAGGCACCA GACCACCTAT TTAAGCGTTC CCATAGTACC GCCTGCTGGC

10201  GGGTTCGAGC CCCGTATCCG GCCGTCCGCC GTGATCCATG CGGTTACCGC CCGCGTGTCG AACCCAGGTG TGCGACGTCA GACAACGGGG GAGTGCTCCT
       CCCAAGCTCG GGGCATAGGC CGGCAGGCGG CACTAGGTAC GCCAATGGCG GGCGCACAGC TTGGGTCCAC ACGCTGCAGT CTGTTGCCCC CTCACGAGGA

10301  TTTGGCTTCC TTCCAGGCGC GGCGGCTGCT GCGCTAGCTT TTTTGGCCAC TGGCCGCGCG CAGCGTAAGC GGTTAGGCTG GAAAGCGAAA GCATTAAGTG
       AAACCGAAGG AAGGTCCGCG CCGCCGACGA CGCGATCGAA AAAACCGGTG ACCGGCGCGC GTCGCATTCG CCAATCCGAC CTTTCGCTTT CGTAATTCAC

10401  GCTCGCTCCC TGTAGCCGGA GGGTTATTTT CCAAGGGTTG AGTCGCGGGA CCCCCGGTTC GAGTCTCGGA CCGGCCGGAC TGCGGCGAAC GGGGGTTTGT
       CGAGCGAGGG ACATCGGCCT CCCAATAAAA GGTTCCCAAC TCAGCGCCCT GGGGGCCAAG CTCAGAGCCT GGCCGGCCTG ACGCCGCTTG CCCCCAAACA

10501  CTCCCCGTCA TGCAAGACCC CGCTTGCAAA TTCCTCCGGA AACAGGGACG AGCCCCTTTT TTGCTTTTCC CAGATGCATC CGGTGCTGCG GCAGATGCGG
       GAGGGGCAGT ACGTTCTGGG GCGAACGTTT AAGGAGGCCT TTGTCCCTGC TCGGGGAAAA AACGAAAAGG GTCTACGTAG GCCACGACGC CGTCTACGCC

10601  CCCCCTCCTC AGCAGCGGCA AGAGCAAGAG CAGCGGCAGA CATGCAGGGC ACCCTCCCCT CCTCCTACCG CGTCAGGAGG GGCGACATCC GCGGTTGACG
       GGGGGAGGAG TCGTCGCCGT TCTCGTTCTC GTCGCCGTCT GTACGTCCCG TGGGAGGGGA GGAGGATGGC GCAGTCCTCC CCGCTGTAGG CGCCAACTGC

10701  CGGCAGCAGA TGGTGATTAC GAACCCCCGC GGCGCCGGGC CCGGCACTAC CTGGACTTGG AGGAGGGCGA GGGCCTGGCG CGGCTAGGAG CGCCCTCTCC
       GCCGTCGTCT ACCACTAATG CTTGGGGGCG CCGCGGCCCG GGCCGTGATG GACCTGAACC TCCTCCCGCT CCCGGACCGC GCCGATCCTC GCGGGAGAGG

10801  TGAGCGGCAC CCAAGGGTGC AGCTGAAGCG TGATACGCGT GAGGCGTACG TGCCGCGGCA GAACCTGTTT CGCGACCGCG AGGGAGAGGA GCCCGAGGAG
       ACTCGCCGTG GGTTCCCACG TCGACTTCGC ACTATGCGCA CTCCGCATGC ACGGCGCCGT CTTGGACAAA GCGCTGGCGC TCCCTCTCCT CGGGCTCCTC

10901  ATGCGGGATC GAAAGTTCCA CGCAGGGCGC GAGCTGCGGC ATGGCCTGAA TCGCGAGCGG TTGCTGCGCG AGGAGGACTT TGAGCCCGAC GCGCGAACCG
       TACGCCCTAG CTTTCAAGGT GCGTCCCGCG CTCGACGCCG TACCGGACTT AGCGCTCGCC AACGACGCGC TCCTCCTGAA ACTCGGGCTG CGCGCTTGGC
                                                                                                 HindIII
11001  GGATTAGTCC CGCGCGCGCA CACGTGGCGG CCGCCGACCT GGTAACCGCA TACGAGCAGA CGGTGAACCA GGAGATTAAC TTTCAAAAAA GCTTTAACAA
       CCTAATCAGG GCGCGCGCGT GTGCACCGCC GGCGGCTGGA CCATTGGCGT ATGCTCGTCT GCCACTTGGT CCTCTAATTG AAAGTTTTTT CGAAATTGTT

11101  CCACGTGCGT ACGCTTGTGG CGCGCGAGGA GGTGGCTATA GGACTGATGC ATCTGTGGGA CTTTGTAAGC GCGCTGGAGC AAAACCCAAA TAGCAAGCCG
       GGTGCACGCA TGCGAACACC GCGCGCTCCT CCACCGATAT CCTGACTACG TAGACACCCT GAAACATTCG CGCGACCTCG TTTTGGGTTT ATCGTTCGGC

11201  CTCATGGCGC AGCTGTTCCT TATAGTGCAG CACAGCAGGG ACAACGAGGC ATTCAGGGAT GCGCTGCTAA ACATAGTAGA GCCCGAGGGC CGCTGGCTGC
       GAGTACCGCG TCGACAAGGA ATATCACGTC GTGTCGTCCC TGTTGCTCCG TAAGTCCCTA CGCGACGATT TGTATCATCT CGGGCTCCCG GCGACCGACG
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Figure 15G

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                     PstI
11301  TCGATTTGAT AAACATCCTG CAGAGCATAG TGGTGCAGGA GCGCAGCTTG AGCCTGGCTG ACAAGGTGGC CGCCATCAAC TATTCCATGC TTAGCCTGGG
       AGCTAAACTA TTTGTAGGAC GTCTCGTATC ACCACGTCCT CGCGTCGAAC TCGGACCGAC TGTTCCACCG GCGGTAGTTG ATAAGGTACG AATCGGACCC
11401  CAAGTTTTAC GCCCGCAAGA TATACCATAC CCCTTACGTT CCCATAGACA AGGAGGTAAA GATCGAGGGG TTCTACATGC GCATGGCGCT GAAGGTGCTG
       GTTCAAAATG CGGGCGTTCT ATATGGTATG GGGAATGCAA GGGTATCTGT TCCTCCATTT CTAGCTCCCC AAGATGTACG CGTACCGCGA CTTCCACGAC
11501  ACCTTGAGCG ACGACCTGGG CGTTTATCGC AACGAGCGCA TCCACAAGGC CGTGAGCGTG AGCCGGCGGC GCGAGCTCAG CGACCGCGAG CTGATGCACA
       TGGAACTCGC TGCTGGACCC GCAAATAGCG TTGCTCGCGT AGGTGTTCCG GCACTCGCAC TCGGCCGCCG CGCTCGAGTC GCTGGCGCTC GACTACGTGT
11601  GCCTGCAAAG GCCCCTGGCT GGCACGGGCA GCGGCGATAG AGAGGCCGAG TCCTACTTTG ACGCGGGCGC TGACCTGCGC TGGGCCCCAA GCCGACGCGC
       CGGACGTTTC CGGGGACCGA CCGTGCCCGT CGCCGCTATC TCTCCGGCTC AGGATGAAAC TGCGCCCGCG ACTGGACGCG ACCCGGGGTT CGGCTGCGCG
11701  CCTGGAGGCA GCTGGGGCCG GACCTGGGCT GGCGGTGGCA CCCGCGCGCG CTGGCAACGT CGGCGGCGTG GAGGAATATG ACGAGGACGA TGAGTACGAG
       GGACCTCCGT CGACCCCGGC CTGGACCCGA CCGCCACCGT GGGCGCGCGC GACCGTTGCA GCCGCCGCAC CTCCTTATAC TGCTCCTGCT ACTCATGCTC
                                                                                                 PstI
11801  CCAGAGGACG GCGAGTACTA AGCGGTGATG TTTCTGATCA GATGATGCAA GACGCAACGG ACCCGGCGGT GCGGGCGGCG CTGCAGAGCC AGCCGTCCGG
       GGTCTCCTGC CGCTCATGAT TCGCCACTAC AAAGACTAGT CTACTACGTT CTGCGTTGCC TGGGCCGCCA CGCCCGCCGC GACGTCTCGG TCGGCAGGCC
11901  CCTTAACTCC ACGGACGACT GGCGCCAGGT CATGGACCGC ATCATGTCGC TGACTGCGCG CAATCCTGAC GCGTTCCGGC AGCAGCCGCA GGCCAACCGG
       GGAATTGAGG TGCCTGCTGA CCGCGGTCCA GTACCTGGCG TAGTACAGCG ACTGACGCGC GTTAGGACTG CGCAAGGCCG TCGTCGGCGT CCGGTTGGCC
                                                                               PvuII
12001  CTCTCCGCAA TTCTGGAAGC GGTGGTCCCG GCGCGCGCAA ACCCCACGCA CGAGAAGGTG CTGGCGATCG TAAACGCGCT GGCCGAAAAC AGGGCCATCC
       GAGAGGCGTT AAGACCTTCG CCACCAGGGC CGCGCGCGTT TGGGGTGCGT GCTCTTCCAC GACCGCTAGC ATTTGCGCGA CCGGCTTTTG TCCCGGTAGG
12101  GGCCCGACGA GGCCGGCCTG GTCTACGACG CGCTGCTTCA GCGCGTGGCT CGTTACAACA GCGGCAACGT GCAGACCAAC CTGGACCGGC TGGTGGGGGA
       CCGGGCTGCT CCGGCCGGAC CAGATGCTGC GCGACGAAGT CGCGCACCGA GCAATGTTGT CGCCGTTGCA CGTCTGGTTG GACCTGGCCG ACCACCCCCT
12201  TGTGCGCGAG GCCGTGGCGC AGCGTGAGCG CGCGCAGCAG CAGGGCAACC TGGGCTCCAT GGTTGCACTA AACGCCTTCC TGAGTACACA GCCCGCCAAC
       ACACGCGCTC CGGCACCGCG TCGCACTCGC GCGCGTCGTC GTCCCGTTGG ACCCGAGGTA CCAACGTGAT TTGCGGAAGG ACTCATGTGT CGGGCGGTTG
12301  GTGCCGCGGG GACAGGAGGA CTACACCAAC TTTGTGAGCG CACTGCGGCT AATGGTGACT GAGACACCGC AAAGTGAGGT GTACCAGTCT GGGCCAGACT
       CACGGCGCCC CTGTCCTCCT GATGTGGTTG AAACACTCGC GTGACGCCGA TTACCACTGA CTCTGTGGCG TTTCACTCCA CATGGTCAGA CCCGGTCTGA
                               PstI
12401  ATTTTTTCCA GACCAGTAGA CAAGGCCTGC AGACCGTAAA CCTGAGCCAG GCTTTCAAAA ACTTGCAGGG GCTGTGGGGG GTGCGGGCTC CCACAGGCGA
       TAAAAAAGGT CTGGTCATCT GTTCCGGACG TCTGGCATTT GGACTCGGTC CGAAAGTTTT TGAACGTCCC CGACACCCCC CACGCCCGAG GGTGTCCGCT
12501  CCGCGCGACC GTGTCTAGCT TGCTGACGCC CAACTCGCGC CTGTTGCTGC TGCTAATAGC GCCCTTCACG GACAGTGGCA GCGTGTCCCG GGACACATAC
       GGCGCGCTGG CACAGATCGA ACGACTGCGG GTTGAGCGCG GACAACGACG ACGATTATCG CGGGAAGTGC CTGTCACCGT CGCACAGGGC CCTGTGTATG
12601  CTAGGTCACT TGCTGACACT GTACCGCGAG GCCATAGGTC AGGCGCATGT GGACGAGCAT ACTTTCCAGG AGATTACAAG TGTCAGCCGC GCGCTGGGGC
       GATCCAGTGA ACGACTGTGA CATGGCGCTC CGGTATCCAG TCCGCGTACA CCTGCTCGTA TGAAAGGTCC TCTAATGTTC ACAGTCGGCG CGCGACCCCG
                                                                                            PmeI
12701  AGGAGGACAC GGGCAGCCTG GAGGCAACCC TAAACTACCT GCTGACCAAC CGGCGGCAGA AGATCCCCTC GTTGCACAGT TTAAACAGCG AGGAGGAGCG
       TCCTCCTGTG CCCGTCGGAC CTCCGTTGGG ATTTGATGGA CGACTGGTTG GCCGCCGTCT TCTAGGGGAG CAACGTGTCA AATTTGTCGC TCCTCCTCGC
12801  CATTTTGCGC TACGTGCAGC AGAGCGTGAG CCTTAACCTG ATGCGCGACG GGGTAACGCC CAGCGTGGCG CTGGACATGA CCGCGCGCAA CATGGAACCG
       GTAAAACGCG ATGCACGTCG TCTCGCACTC GGAATTGGAC TACGCGCTGC CCCATTGCGG GTCGCACCGC GACCTGTACT GGCGCGCGTT GTACCTTGGC
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Figure 15H

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12901  GGCATGTATG CCTCAAACCG GCCGTTTATC AACCGCCTAA TGGACTACTT GCATCGCGCG GCCGCCGTGA ACCCCGAGTA TTTCACCAAT GCCATCTTGA
       CCGTACATAC GGAGTTTGGC CGGCAAATAG TTGGCGGATT ACCTGATGAA CGTAGCGCGC CGGCGGCACT TGGGGCTCAT AAAGTGGTTA CGGTAGAACT
13001  ACCCGCACTG GCTACCGCCC CCTGGTTTCT ACACCGGGGG ATTCGAGGTG CCTGAGGGTA ACGATGGATT CCTCTGGGAC GACATAGACG ACAGCGTGTT
       TGGGCGTGAC CGATGGCGGG GGACCAAAGA TGTGGCCCCC TAAGCTCCAC GGACTCCCAT TGCTACCTAA GGAGACCCTG CTGTATCTGC TGTCGCACAA
                                                                                   HindIII
13101  TTCCCCGCAA CCGCAGACCC TGCTAGAGTT GCAACAGCGC GAGCAGGCAG AGGCGGCGCT GCGAAAGGAA AGCTTCCGCA GGCCAAGCAG CTTGTCCGAT
       AAGGGGCGTT GGCGTCTGGG ACGATCTCAA CGTTGTCGCG CTCGTCCGTC TCCGCCGCGA CGCTTTCCTT TCGAAGGCGT CCGGTTCGTC GAACAGGCTA
                                                HindIII
13201  CTAGGCGCTG CGGCCCCGCG GTCAGATGCT AGTAGCCCAT TTCCAAGCTT GATAGGGTCT CTTACCAGCA CTCGCACCAC CCGCCCGCGC CTGCTGGGC
       GATCCGCGAC GCCGGGGCGC CAGTCTACGA TCATCGGGTA AAGGTTCGAA CTATCCCAGA GAATGGTCGT GAGCGTGGTG GGCGGGCGCG GACGACCCG
                              PstI
13301  AGGAGGAGTA CCTAAACAAC TCGCTGCTGC AGCCGCAGCG CGAAAAAAAC CTGCCTCCGG CATTTCCCAA CAACGGGATA GAGAGCCTAG TGGACAAGAT
       TCCTCCTCAT GGATTTGTTG AGCGACGACG TCGGCGTCGC GCTTTTTTTG GACGGAGGCC GTAAAGGGTT GTTGCCCTAT CTCTCGGATC ACCTGTTCTA
13401  GAGTAGATGG AAGACGTACG CGCAGGAGCA CAGGGACGTG CCAGGCCCGC GCCCGCCCAC CCGTCGTCAA AGGCACGACC GTCAGCGGGG TCTGGTGTGG
       CTCATCTACC TTCTGCATGC GCGTCCTCGT GTCCCTGCAC GGTCCGGGCG CGGGCGGGTG GGCAGCAGTT TCCGTGCTGG CAGTCGCCCC AGACCACACC
13501  GAGGACGATG ACTCGGCAGA CGACAGCAGC GTCCTGGATT TGGGAGGGAG TGGCAACCCG TTTGCGCACC TTCGCCCCAG GCTGGGGAGA ATGTTTTAAA
       CTCCTGCTAC TGAGCCGTCT GCTGTCGTCG CAGGACCTAA ACCCTCCCTC ACCGTTGGGC AAACGCGTGG AAGCGGGGTC CGACCCCTCT TACAAAATTT
13601  AAAAAAAAAA GCATGATGCA AAATAAAAAA CTCACCAAGG CCATGGCACC GAGCGTTGGT TTTCTTGTAT TCCCCTTAGT ATGCGGCGCG CGGCGATGTA
       TTTTTTTTTT CGTACTACGT TTTATTTTTT GAGTGGTTCC GGTACCGTGG CTCGCAACCA AAAGAACATA AGGGGAATCA TACGCCGCGC GCCGCTACAT
13701  TGAGGAAGGT CCTCCTCCCT CCTACGAGAG TGTGGTGAGC GCGGCGCCAG TGGCGGCGGC GCTGGGTTCT CCCTTCGATG CTCCCCTGGA CCCGCCGTTT
       ACTCCTTCCA GGAGGAGGGA GGATGCTCTC ACACCACTCG CGCCGCGGTC ACCGCCGCCG CGACCCAAGA GGGAAGCTAC GAGGGGACCT GGGCGGCAA..
                  KpnI
13801  GTGCCTCCGC GGTACCTGCG GCCTACCGGG GGGAGAAACA GCATCCGTTA CTCTGAGTTG GCACCCCTAT TCGACACCAC CCGTGTGTAC CTGGTGGACA
       CACGGAGGCG CCATGGACGC CGGATGGCCC CCCTCTTTGT CGTAGGCAAT GAGACTCAAC CGTGGGGATA AGCTGTGGTG GGCACACATG GACCACCTGT
13901  ACAAGTCAAC GGATGTGGCA TCCCTGAACT ACCAGAACGA CCACAGCAAC TTTCTGACCA CGGTCATTCA AAACAATGAC TACAGCCCGG GGGAGGCAAG
       TGTTCAGTTG CCTACACCGT AGGGACTTGA TGGTCTTGCT GGTGTCGTTG AAAGACTGGT GCCAGTAAGT TTTGTTACTG ATGTCGGGCC CCCTCCGTTC
14001  CACACAGACC ATCAATCTTG ACGACCGGTC GCACTGGGGC GGCGACCTGA AAACCATCCT GCATACCAAC ATGCCAAATG TGAACGAGTT CATGTTTACC
       GTGTGTCTGG TAGTTAGAAC TGCTGGCCAG CGTGACCCCG CCGCTGGACT TTTGGTAGGA CGTATGGTTG TACGGTTTAC ACTTGCTCAA GTACAAATGG
14101  AATAAGTTTA AGGCGCGGGT GATGGTGTCG CGCTTGCCTA CTAAGGACAA TCAGGTGGAG CTGAAATACG AGTGGGTGGA GTTCACGCTG CCCGAGGGCA
       TTATTCAAAT TCCGCGCCCA CTACCACAGC GCGAACGGAT GATTCCTGTT AGTCCACCTC GACTTTATGC TCACCCACCT CAAGTGCGAC GGGCTCCCGT
                                                   PvuI
14201  ACTACTCCGA GACCATGACC ATAGACCTTA TGAACAACGC GATCGTGGAG CACTACTTGA AAGTGGGCAG ACAGAACGGG GTTCTGGAAA GCGACATCGG
       TGATGAGGCT CTGGTACTGG TATCTGGAAT ACTTGTTGCG CTAGCACCTC GTGATGAACT TTCACCCGTC TGTCTTGCCC CAAGACCTTT CGCTGTAGCC
14301  GGTAAAGTTT GACACCCGCA ACTTCAGACT GGGGTTTGAC CCCGTCACTG GTCTTGTCAT GCCTGGGGTA TATACAAACG AAGCCTTCCA TCCAGACATC
       CCATTTCAAA CTGTGGGCGT TGAAGTCTGA CCCCAAACTG GGGCAGTGAC CAGAACAGTA CGGACCCCAT ATATGTTTGC TTCGGAAGGT AGGTCTGTAG
14401  ATTTTGCTGC CAGGATGCGG GGTGGACTTC ACCCACAGCC GCCTGAGCAA CTTGTTGGGC ATCCGCAAGC GGCAACCCTT CCAGGAGGGC TTTAGGATCA
       TAAAACGACG GTCCTACGCC CCACCTGAAG TGGGTGTCGG CGGACTCGTT GAACAACCCG TAGGCGTTCG CCGTTGGGAA GGTCCTCCCG AAATCCTAGT
...
```

Figure 15I

pMRKAd5gag MER6R2

14501 CCTACGATGA TCTGGAGGGT GGTAACATTC CCGCACTGTT GGATGTGGAC GCCTACCAGG CGAGCTTGAA AGATGACACC GAACAGGGCG GGGGTGGCGC
GGATGCTACT AGACCTCCCA CCATTGTAAG GGCGTGACAA CCTACACCTG CGGATGGTCC GCTCGAACTT TCTACTGTGG CTTGTCCCGC CCCCACCGCG

14601 AGGCGGCAGC AACAGCAGTG GCAGCGGCGC GGAAGAGAAC TCCAACGCGG CAGCGGCGGC AATGCAGCCG GTGGAGGACA TGAACGATCA TGCCATTCGC
TCCGCCGTCG TTGTCGTCAC CGTCGCCGCG CCTTCTCTTG AGGTTGCGCC GTCGCCGCCG TTACGTCGGC CACCTCCTGT ACTTGCTAGT ACGGTAAGCG

14701 GGCGACACCT TTGCCACACG GGCTGAGGAG AAGCGCGCTG AGGCCGAAGC AGCGGCCGAA GCTGCCGCCC CCGCTGCGCA ACCCGAGGTC GAGAAGCCTC
CCGCTGTGGA AACGGTGTGC CCGACTCCTC TTCGCGCGAC TCCGGCTTCG TCGCCGGCTT CGACGGCGGG GGCGACGCGT TGGGCTCCAG CTCTTCGGAG
KpnI

14801 AGAAGAAACC GGTGATCAAA CCCCTGACAG AGGACAGCAA GAAACGCAGT TACAACCTAA TAAGCAATGA CAGCACCTTC ACCCAGTACC GCAGCTGGTA
TCTTCTTTGG CCACTAGTTT GGGGACTGTC TCCTGTCGTT CTTTGCGTCA ATGTTGGATT ATTCGTTACT GTCGTGGAAG TGGGTCATGG CGTCGACCAT
Kpn

14901 CCTTGCATAC AACTACGGCG ACCCTCAGAC CGGAATCCGC TCATGGACCC TGCTTTGCAC TCCTGACGTA ACCTGCGGCT CGGAGCAGGT CTACTGGTCG
GGAACGTATG TTGATGCCGC TGGGAGTCTG GCCTTAGGCG AGTACCTGGG ACGAAACGTG AGGACTGCAT TGGACGCCGA GCCTCGTCCA GATGACCAGC

15001 TTGCCAGACA TGATGCAAGA CCCCGTGACC TTCCGCTCCA CGCGCCAGAT CAGCAACTTT CCGGTGGTGG GCGCCGAGCT GTTGCCCGTG CACTCCAAGA
AACGGTCTGT ACTACGTTCT GGGGCACTGG AAGGCGAGGT GCGCGGTCTA GTCGTTGAAA GGCCACCACC CGCGGCTCGA CAACGGGCAC GTGAGGTTCT
AscI

15101 GCTTCTACAA CGACCAGGCC GTCTACTCCC AACTCATCCG CCAGTTTACC TCTCTGACCC ACGTGTTCAA TCGCTTTCCC GAGAACCAGA TTTTGGCGCG
CGAAGATGTT GCTGGTCCGG CAGATGAGGG TTGAGTAGGC GGTCAAATGG AGAGACTGGG TGCACAAGTT AGCGAAAGGG CTCTTGGTCT AAAACCGCGC
AscI

15201 CCCGCCAGCC CCCACCATCA CCACCGTCAG TGAAAACGTT CCTGCTCTCA CAGATCACGG GACGCTACCG CTGCGCAACA GCATCGGAGG AGTCCAGCGA
GGGCGGTCGG GGGTGGTAGT GGTGGCAGTC ACTTTTGCAA GGACGAGAGT GTCTAGTGCC CTGCGATGGC GACGCGTTGT CGTAGCCTCC TCAGGTCGCT

15301 GTGACCATTA CTGACGCCAG ACGCCGCACC TGCCCCTACG TTTACAAGGC CCTGGGCATA GTCTCGCCGC GCGTCCTATC GAGCCGCACT TTTTGAGCAA
CACTGGTAAT GACTGCGGTC TGCGGCGTGG ACGGGGATGC AAATGTTCCG GGACCCGTAT CAGAGCGGCG CGCAGGATAG CTCGGCGTGA AAAACTCGTT

15401 GCATGTCCAT CCTTATATCG CCCAGCAATA ACACAGGCTG GGGCCTGCGC TTCCCAAGCA AGATGTTTGG CGGGGCCAAG AAGCGCTCCG ACCAACACCC
CGTACAGGTA GGAATATAGC GGGTCGTTAT TGTGTCCGAC CCCGGACGCG AAGGGTTCGT TCTACAAACC GCCCCGGTTC TTCGCGAGGC TGGTTGTGGG

15501 AGTGCGCGTG CGCGGGCACT ACCGCGCGCC CTGGGGCGCG CACAAACGCG GCCGCACTGG GCGCACCACC GTCGATGACG CCATCGACGC GGTGGTGGAG
TCACGCGCAC GCGCCCGTGA TGGCGCGCGG GACCCCGCGC GTGTTTGCGC CGGCGTGACC CGCGTGGTGG CAGCTACTGC GGTAGCTGCG CCACCACCTC

15601 GAGGCGCGCA ACTACACGCC CACGCCGCCA CCAGTGTCCA CAGTGGACGC GGCCATTCAG ACCGTGGTGC GCGGAGCCCG GCGCTATGCT AAAATGAAGA
CTCCGCGCGT TGATGTGCGG GTGCGGCGGT GGTCACAGGT GTCACCTGCG CCGGTAAGTC TGGCACCACG CGCCTCGGGC CGCGATACGA TTTTACTTCT

15701 GACGGCGGAG GCGCGTAGCA CGTCGCCACC GCCGCCGACC CGGCACTGCC GCCCAACGCG CGGCGGCGGC CCTGCTTAAC CGCGCACGTC GCACCGGCCG
CTGCCGCCTC CGCGCATCGT GCAGCGGTGG CGGCGGCTGG GCCGTGACGG CGGGTTGCGC GCCGCCGCCG GGACGAATTG GCGCGTGCAG CGTGGCCGGC
SfiI

15801 ACGGGCGGCC ATGCGGGCCG CTCGAAGGCT GGCCGCGGGT ATTGTCACTG TGCCCCCCAG GTCCAGGCGA CGAGCGGCCG CCGCAGCAGC CGCGGCCATT
TGCCCGCCGG TACGCCCGGC GAGCTTCCGA CCGGCGCCCA TAACAGTGAC ACGGGGGGTC CAGGTCCGCT GCTCGCCGGC GGCGTCGTCG GCGCCGGTAA

15901 AGTGCTATGA CTCAGGGTCG CAGGGGCAAC GTGTATTGGG TGCGCGACTC GGTTAGCGGC CTGCGCGTGC CCGTGCGCAC CCGCCCCCCG CGCAACTAGA
TCACGATACT GAGTCCCAGC GTCCCCGTTG CACATAACCC ACGCGCTGAG CCAATCGCCG GACGCGCACG GGCACGCGTG GGCGGGGGGC GCGTTGATCT

16001 TTGCAAGAAA AAACTACTTA GACTCGTACT GTTGTATGTA TCCAGCGGCG GCGGCGCGCA ACGAAGCTAT GTCCAAGCGC AAAATCAAAG AAGAGATGCT
AACGTTCTTT TTTGATGAAT CTGAGCATGA CAACATACAT AGGTCGCCGC CGCCGCGCGT TGCTTCGATA CAGGTTCGCG TTTTAGTTTC TTCTCTACGA

Figure 15J

pMRKAd5gag MER6B2

BglII

16101 CCAGGTCATC GCGCCGGAGA TCTATGGCCC CCCGAAGAAG GAAGAGCAGG ATTACAAGCC CCGAAAGCTA AAGCGGGTCA AAAAGAAAAA GAAAGATGAT
GGTCCAGTAG CGCGGCCTCT AGATACCGGG GGGCTTCTTC CTTCTCGTCC TAATGTTCGG GGCTTTCGAT TTCGCCCAGT TTTTCTTTTT CTTTCTACT

SalI

16201 GATGATGAAC TTGACGACGA GGTGGAACTG CTGCACGCTA CCGCGCCCAG GCGACGGGTA CAGTGGAAAG GTCGACGCGT AAAACGTGTT TTGCGACCC
CTACTACTTG AACTGCTGCT CCACCTTGAC GACGTGCGAT GGCGCGGGTC CGCTGCCCAT GTCACCTTTC CAGCTGCGCA TTTTGCACAA AACGCTGGG

16301 GCACCACCGT AGTCTTTACG CCCGGTGAGC GCTCCACCCG CACCTACAAG CGCGTGTATG ATGAGGTGTA CGGCGACGAG GACCTGCTTG AGCAGGCCAA
CGTGGTGGCA TCAGAAATGC GGGCCACTCG CGAGGTGGGC GTGGATGTTC GCGCACATAC TACTCCACAT GCCGCTGCTC CTGGACGAAC TCGTCCGGTT

16401 CGAGCGCCTC GGGGAGTTTG CCTACGGAAA GCGGCATAAG GACATGCTGG CGTTGCCGCT GGACGAGGGC AACCCAACAC CTAGCCTAAA GCCCGTAACA
GCTCGCGGAG CCCCTCAAAC GGATGCCTTT CGCCGTATTC CTGTACGACC GCAACGGCGA CCTGCTCCCG TTGGGTTGTG GATCGGATTT CGGGCATTGT

PstI KpnI

16501 CTGCAGCAGG TGCTGCCCGC GCTTGCACCG TCCGAAGAAA AGCGCGGCCT AAAGCGCGAG TCTGGTGACT TGGCACCCAC CGTGCAGCTG ATGGTACCCA
GACGTCGTCC ACGACGGGCG CGAACGTGGC AGGCTTCTTT TCGCGCCGGA TTTCGCGCTC AGACCACTGA ACCGTGGGTG GCACGTCGAC TACCATGGGT

16601 AGCCCCAGCG ACTGGAAGAT GTCTTGGAAA AAATGACCGT GGAACCTGGG CTGGAGCCCG AGGTCCGCGT GCGGCCAATC AAGCAGGTGG CGCCGGGACT
TCGGGGTCGC TGACCTTCTA CAGAACCTTT TTTACTGGCA CCTTGGACCC GACCTCGGGC TCCAGGCGCA CGCCGGTTAG TTCGTCCACC GCGGCCCTGA

16701 GGGCGTGCAG ACCGTGGACG TTCAGATACC CACTACCAGT AGCACCAGTA TTGCCACCGC CACAGAGGGC ATGGAGACAC AAACGTCCCC GGTTGCCTCA
CCCGCACGTC TGGCACCTGC AAGTCTATGG GTGATGGTCA TCGTGGTCAT AACGGTGGCG GTGTCTCCCG TACCTCTGTG TTTGCAGGGG CCAACGGAGT

16801 GCGGTGGCGG ATGCCGCGGT GCAGGCGGTC GCTGCGGCCG CGTCCAAGAC CTCTACGGAG GTGCAAACGG ACCCGTGGAT GTTTCGCGTT TCAGCCCCCC
CGCCACCGCC TACGGCGCCA CGTCCGCCAG CGACGCCGGC GCAGGTTCTG GAGATGCCTC CACGTTTGCC TGGGCACCTA CAAAGCGCAA AGTCGGGGGG

16901 GGCGCCCGCG CCGTTCGAGG AAGTACGGCG CCGCCAGCGC GCTACTGCCC GAATATGCCC TACATCCTTC CATTGCGCCT ACCCCCGGCT ATCGTGGCT
CCGCGGGCGC GGCAAGCTCC TTCATGCCGC GGCGGTCGCG CGATGACGGG CTTATACGGG ATGTAGGAAG GTAACGCGGA TGGGGGCCGA TAGCACCGA

17001 CACCTACCGC CCCAGAAGAC GAGCAACTAC CCGACGCCGA ACCACCACTG GAACCCGCCG CCGCCGTCGC CGTCGCCAGC CCGTGCTGGC CCCGATTTCC
GTGGATGGCG GGGTCTTCTG CTCGTTGATG GGCTGCGGCT TGGTGGTGAC CTTGGGCGGC GGCGGCAGCG GCAGCGGTCG GGCACGACCG GGGCTAAAGG

17101 GTGCGCAGGG TGGCTCGCGA AGGAGGCAGG ACCCTGGTGC TGCCAACAGC GCGCTACCAC CCCAGCATCG TTTAAAAGCC GGTCTTTGTG GTTCTTGCAG
CACGCGTCCC ACCGAGCGCT TCCTCCGTCC TGGGACCACG ACGGTTGTCG CGCGATGGTG GGGTCGTAGC AAATTTTCGG CCAGAAACAC CAAGAACGTC

SphI

17201 ATATGGCCCT CACCTGCCGC CTCCGTTTCC CGGTGCCGGG ATTCCGAGGA AGAATGCACC GTAGGAGGGG CATGGCCGGC CACGGCCTGA CGGGCGGCAT
TATACCGGGA GTGGACGGCG GAGGCAAAGG GCCACGGCCC TAAGGCTCCT TCTTACGTGG CATCCTCCCC GTACCGGCCG GTGCCGGACT GCCCGCCGTA

Sph SphI

17301 GCGTCGTGCG CACCACCGGC GGCGGCGCGC GTCGCACCGT CGCATGCGCG GCGGTATCCT GCCCCTCCTT ATTCCACTGA TCGCCGCGGC GATTGGCGCC
CGCAGCACGC GTGGTGGCCG CCGCCGCGCG CAGCGTGGCA GCGTACGCGC CGCCATAGGA CGGGGAGGAA TAAGGTGACT AGCGGCGCCG CTAACCGCGG

17401 GTGCCCGGAA TTGCATCCGT GGCCTTGCAG GCGCAGAGAC ACTGATTAAA AACAAGTTGC ATGTGGAAAA ATCAAAATAA AAAGTCTGGA CTCTCACGCT
CACGGGCCTT AACGTAGGCA CCGGAACGTC CGCGTCTCTG TGACTAATTT TTGTTCAACG TACACCTTTT TAGTTTTATT TTTCAGACCT GAGAGTGCGA

EcoRV

17501 CGCTTGGTCC TGTAACTATT TTGTAGAATG GAAGACATCA ACTTTGCGTC TCTGGCCCCG CGACACGGCT CGCGCCCGTT CATGGGAAAC TGGCAAGATA
GCGAACCAGG ACATTGATAA AACATCTTAC CTTCTGTAGT TGAAACGCAG AGACCGGGGC GCTGTGCCGA GCGCGGGCAA GTACCCTTTG ACCGTTCTAT

Figure 15K

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      EcoRV
17601 TCGGCACCAG CAATATGAGC GGTGGCGCCT TCAGCTGGGG CTCGCTGTGG AGCGGCATTA AAAATTTCGG TTCCACCGTT AAGAACTATG GCAGCAAGGC
      AGCCGTGGTC GTTATACTCG CCACCGCGGA AGTCGACCCC GAGCGACACC TCGCCGTAAT TTTTAAAGCC AAGGTGGCAA TTCTTGATAC CGTCGTTCCG
17701 CTGGAACAGC AGCACAGGCC AGATGCTGAG GGATAAGTTG AAAGAGCAAA ATTTCCAACA AAACGTGGTA GATGGCCTGG CCTCTGGCAT TAGCGGGGTG
      GACCTTGTCG TCGTGTCCGG TCTACGACTC CCTATTCAAC TTTCTCGTTT TAAAGGTTGT TTTGCACCAT CTACCGGACC GGAGACCGTA ATCGCCCCAC
                                                HindIII
17801 GTGGACCTGG CCAACCAGGC AGTGCAAAAT AAGATTAACA GTAAGCTTGA TCCCCGCCCT CCCGTAGAGG AGCCTCCACC GGCCGTGGAG ACAGTGTCTC
      CACCTGGACC GGTTGGTCCG TCACGTTTTA TTCTAATTGT CATTCGAACT AGGGGCGGGA GGGCATCTCC TCGGAGGTGG CCGGCACCTC TGTCACAGAG
17901 CAGAGGGGCG TGGCGAAAAG CGTCCGGCCC CCGACAGGGA AGAAACTCTG GTGACGCAAA TAGACGAGCC TCCCTCGTAC GAGGAGGCAC TAAAGCAAGG
      GTCTCCCCGC ACCGCTTTTC GCAGGCCGGG GGCTGTCCCT TCTTTGAGAC CACTGCGTTT ATCTGCTCGG AGGGAGCATG CTCCTCCGTG ATTTCGTTCC
18001 CCTGCCCACC ACCCGTCCCA TCGCGCCCAT GGCTACCGGA GTGCTGGGCC AGCACACACC CGTAACGCTG GACCTGCCTC CCCCCGCCGA CACCCAGCAG
      GGACGGGTGG TGGGCAGGGT AGCGCGGGTA CCGATGGCCT CACGACCCGG TCGTGTGTGG GCATTGCGAC CTGGACGGAG GGGGGCGGCT GTGGGTCGTC
                                                                                            PvuI
18101 AAACCTGTGC TGCCAGGCCC GACCGCCGTT GTTGTAACCC GTCCTAGCCG CGCGTCCCTG CGCCGCGCCG CCAGCGGTCC GCGATCGTTG CGGCCCGTAG
      TTTGGACACG ACGGTCCGGG CTGGCGGCAA CAACATTGGG CAGGATCGGC GCGCAGGGAC GCGGCGCGGC GGTCGCCAGG CGCTAGCAAC GCCGGGCATC
18201 CCAGTGGCAA CTGGCAAAGC ACACTGAACA GCATCGTGGG TCTGGGGGTG CAATCCCTGA AGCGCCGACG ATGCTTCTGA TAGCTAACGT GTCGTATGTG
      GGTCACCGTT GACCGTTTCG TGTGACTTGT CGTAGCACCC AGACCCCCAC GTTAGGGACT TCGCGGCTGC TACGAAGACT ATCGATTGCA CAGCATACAC
18301 TGTCATGTAT GCGTCCATGT CGCCGCCAGA GGAGCTGCTG AGCCGCCGCG CGCCCGCTTT CCAAGATGGC TACCCCTTCG ATGATGCCGC AGTGGTCTTA
      ACAGTACATA CGCAGGTACA GCGGCGGTCT CCTCGACGAC TCGGCGGCGC GCGGGCGAAA GGTTCTACCG ATGGGGAAGC TACTACGGCG TCACCAGAAT
18401 CATGCACATC TCGGGCCAGG ACGCCTCGGA GTACCTGAGC CCCGGGCTGG TGCAGTTTGC CCGCGCCACC GAGACGTACT TCAGCCTGAA TAACAAGTTT
      GTACGTGTAG AGCCCGGTCC TGCGGAGCCT CATGGACTCG GGGCCCGACC ACGTCAAACG GGCGCGGTGG CTCTGCATGA AGTCGGACTT ATTGTTCAAA
18501 AGAAACCCCA CGGTGGCGCC TACGCACGAC GTGACCACAG ACCGGTCCCA GCGTTTGACG CTGCGGTTCA TCCCTGTGGA CCGTGAGGAT ACTGCGTACT
      TCTTTGGGGT GCCACCGCGG ATGCGTGCTG CACTGGTGTC TGGCCAGGGT CGCAAACTGC GACGCCAAGT AGGGACACCT GGCACTCCTA TGACGCATGA
18601 CGTACAAGGC GCGGTTCACC CTAGCTGTGG GTGATAACCG TGTGCTGGAC ATGGCTTCCA CGTACTTTGA CATCCGCGGC GTGCTGGACA GGGGCCCTAC
      GCATGTTCCG CGCCAAGTGG GATCGACACC CACTATTGGC ACACGACCTG TACCGAAGGT GCATGAAACT GTAGGCGCCG CACGACCTGT CCCCGGGATG
18701 TTTTAAGCCC TACTCTGGCA CTGCCTACAA CGCCCTGGCT CCCAAGGGTG CCCCAAATCC TTGCGAATGG GATGAAGCTG CTACTGCTCT TGAAATAAAC
      AAAATTCGGG ATGAGACCGT GACGGATGTT GCGGGACCGA GGGTTCCCAC GGGGTTTAGG AACGCTTACC CTACTTCGAC GATGACGAGA ACTTTATTTG
18801 CTAGAAGAAG AGGACGATGA CAACGAAGAC GAAGTAGACG AGCAAGCTGA GCAGCAAAAA ACTCACGTAT TTGGGCAGGC GCCTTATTCT GGTATAAATA
      GATCTTCTTC TCCTGCTACT GTTGCTTCTG CTTCATCTGC TCGTTCGACT CGTCGTTTTT TGAGTGCATA AACCCGTCCG CGGAATAAGA CCATATTTAT
18901 TTACAAAGGA GGGTATTCAA ATAGGTGTCG AAGGTCAAAC ACCTAAATAT GCCGATAAAA CATTTCAACC TGAACCTCAA ATAGGAGAAT CTCAGTGGTA
      AATGTTTCCT CCCATAAGTT TATCCACAGC TTCCAGTTTG TGGATTTATA CGGCTATTTT GTAAAGTTGG ACTTGGAGTT TATCCTCTTA GAGTCACCAT
19001 CGAAACAGAA ATTAATCATG CAGCTGGGAG AGTCCTAAAA AAGACTACCC CAATGAAACC ATGTTACGGT TCATATGCAA AACCCACAAA TGAAAATGGG
      GCTTTGTCTT TAATTAGTAC GTCGACCCTC TCAGGATTTT TTCTGATGGG GTTACTTTGG TACAATGCCA AGTATACGTT TTGGGTGTTT ACTTTTACCC
19101 GGGCAAGGCA TTCTTGTAAA GCAACAAAAT GGAAAGCTAG AAAGTCAAGT GGAAATGCAA TTTTTCTCAA CTACTGAGGC AGCCGCAGGC AATGGTGATA
      CCCGTTCCGT AAGAACATTT CGTTGTTTTA CCTTTCGATC TTTCAGTTCA CCTTTACGTT AAAAAGAGTT GATGACTCCG TCGGCGTCCG TTACCACTAT
19201 ACTTGACTCC TAAAGTGGTA TTGTACAGTG AAGATGTAGA TATAGAAACC CCAGACACTC ATATTTCTTA CATGCCCACT ATTAAGGAAG GTAACTCACG
      TGAACTGAGG ATTTCACCAT AACATGTCAC TTCTACATCT ATATCTTTGG GGTCTGTGAG TATAAAGAAT GTACGGGTGA TAATTCCTTC CATTGAGTGC
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Figure 15L

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19301  AGAACTAATG GGCCAACAAT CTATGCCCAA CAGGCCTAAT TACATTGCTT TTAGGGACAA TTTTATTGGT CTAATGTATT ACAACAGCAC GGGTAATATG
       TCTTGATTAC CCGGTTGTTA GATACGGGTT GTCCGGATTA ATGTAACGAA AATCCCTGTT AAAATAACCA GATTACATAA TGTTGTCGTG CCCATTATAC

19401  GGTGTTCTGG CGGGCCAAGC ATCGCAGTTG AATGCTGTTG TAGATTTGCA AGACAGAAAC ACAGAGCTTT CATACCAGCT TTTGCTTGAT TCCATTGGTG
       CCACAAGACC GCCCGGTTCG TAGCGTCAAC TTACGACAAC ATCTAAACGT TCTGTCTTTG TGTCTCGAAA GTATGGTCGA AAACGAACTA AGGTAACCAC

19501  ATAGAACCAG GTACTTTTCT ATGTGGAATC AGGCTGTTGA CAGCTATGAT CCAGATGTTA GAATTATTGA AAATCATGGA ACTGAAGATG AACTTCCAAA
       TATCTTGGTC CATGAAAAGA TACACCTTAG TCCGACAACT GTCGATACTA GGTCTACAAT CTTAATAACT TTTAGTACCT TGACTTCTAC TTGAAGGTTT

19601  TTACTGCTTT CCACTGGGAG GTGTGATTAA TACAGAGACT CTTACCAAGG TAAAACCTAA AACAGGTCAG GAAAATGGAT GGGAAAAAGA TGCTACAGAA
       AATGACGAAA GGTGACCCTC CACACTAATT ATGTCTCTGA GAATGGTTCC ATTTTGGATT TTGTCCAGTC CTTTTACCTA CCCTTTTTCT ACGATGTCTT

19701  TTTTCAGATA AAAATGAAAT AAGAGTTGGA AATAATTTTG CCATGGAAAT CAATCTAAAT GCCAACCTGT GGAGAAATTT CCTGTACTCC AACATAGCGC
       AAAAGTCTAT TTTTACTTTA TTCTCAACCT TTATTAAAAC GGTACCTTTA GTTAGATTTA CGGTTGGACA CCTCTTTAAA GGACATGAGG TTGTATCGCG

19801  TGTATTTGCC CGACAAGCTA AAGTACAGTC CTTCCAACGT AAAAATTTCT GATAACCCAA ACACCTACGA CTACATGAAC AAGCGAGTGG TGGCTCCCGG
       ACATAAACGG GCTGTTCGAT TTCATGTCAG GAAGGTTGCA TTTTTAAAGA CTATTGGGTT TGTGGATGCT GATGTACTTG TTCGCTCACC ACCGAGGGCC

19901  GCTAGTGGAC TGCTACATTA ACCTTGGAGC ACGCTGGTCC CTTGACTATA TGGACAACGT CAACCCATTT AACCACCACC GCAATGCTGG CCTGCGCTAC
       CGATCACCTG ACGATGTAAT TGGAACCTCG TGCGACCAGG GAACTGATAT ACCTGTTGCA GTTGGGTAAA TTGGTGGTGG CGTTACGACC GGACGCGATG

20001  CGCTCAATGT TGCTGGGCAA TGGTCGCTAT GTGCCCTTCC ACATCCAGGT GCCTCAGAAG TTCTTTGCCA TTAAAAACCT CCTTCTCCTG CCGGGCTCAT
       GCGAGTTACA ACGACCCGTT ACCAGCGATA CACGGGAAGG TGTAGGTCCA CGGAGTCTTC AAGAAACGGT AATTTTTGGA GGAAGAGGAC GGCCCGAGTA

                                               PstI
20101  ACACCTACGA GTGGAACTTC AGGAAGGATG TTAACATGGT TCTGCAGAGC TCCCTAGGAA ATGACCTAAG GGTTGACGGA GCCAGCATTA AGTTTGATAG
       TGTGGATGCT CACCTTGAAG TCCTTCCTAC AATTGTACCA AGACGTCTCG AGGGATCCTT TACTGGATTC CCAACTGCCT CGGTCGTAAT TCAAACTATC

20201  CATTTGCCTT TACGCCACCT TCTTCCCCAT GGCCCACAAC ACCGCCTCCA CGCTTGAGGC CATGCTTAGA AACGACACCA ACGACCAGTC CTTTAACGAC
       GTAAACGGAA ATGCGGTGGA AGAAGGGGTA CCGGGTGTTG TGGCGGAGGT GCGAACTCCG GTACGAATCT TTGCTGTGGT TGCTGGTCAG GAAATTGCTG

20301  TATCTCTCCG CCGCCAACAT GCTCTACCCT ATACCCGCCA ACGCTACCAA CGTGCCCATA TCCATCCCCT CCCGCAACTG GGCGGCTTTC CGCGGCTGGG
       ATAGAGAGGC GGCGGTTGTA CGAGATGGGA TATGGGCGGT TGCGATGGTT GCACGGGTAT AGGTAGGGGA GGGCGTTGAC CCGCCGAAAG GCGCCGACCC

20401  CCTTCACGCG CCTTAAGACT AAGGAAACCC CATCACTGGG CTCGGGCTAC GACCCTTATT ACACCTACTC TGGCTCTATA CCCTACCTAG ATGGAACCTT
       GGAAGTGCGC GGAATTCTGA TTCCTTTGGG GTAGTGACCC GAGCCCGATG CTGGGAATAA TGTGGATGAG ACCGAGATAT GGGATGGATC TACCTTGGAA

20501  TTACCTCAAC CACACCTTTA AGAAGGTGGC CATTACCTTT GACTCTTCTG TCAGCTGGCC TGGCAATGAC CGCCTGCTTA CCCCCAACGA GTTTGAAATT
       AATGGAGTTG GTGTGGAAAT TCTTCCACCG GTAATGGAAA CTGAGAAGAC AGTCGACCGG ACCGTTACTG GCGGACGAAT GGGGGTTGCT CAAACTTTAA

20601  AAGCGCTCAG TTGACGGGGA GGGTTACAAC GTTGCCCAGT GTAACATGAC CAAAGACTGG TTCCTGGTAC AAATGCTAGC TAACTATAAC ATTGGCTACC
       TTCGCGAGTC AACTGCCCCT CCCAATGTTG CAACGGGTCA CATTGTACTG GTTTCTGACC AAGGACCATG TTTACGATCG ATTGATATTG TAACCGATGG

20701  AGGGCTTCTA TATCCCAGAG AGCTACAAGG ACCGCATGTA CTCCTTCTTT AGAAACTTCC AGCCCATGAG CCGTCAGGTG GTGGATGATA CTAAATACAA
       TCCCGAAGAT ATAGGGTCTC TCGATGTTCC TGGCGTACAT GAGGAAGAAA TCTTTGAAGG TCGGGTACTC GGCAGTCCAC CACCTACTAT GATTTATGTT

20801  GGACTACCAA CAGGTGGGCA TCCTACACCA ACACAACAAC TCTGGATTTG TTGGCTACCT TGCCCCCACC ATGCGCGAAG GACAGGCCTA CCCTGCTAAC
       CCTGATGGTT GTCCACCCGT AGGATGTGGT TGTGTTGTTG AGACCTAAAC AACCGATGGA ACGGGGGTGG TACGCGCTTC CTGTCCGGAT GGGACGATTG

                                                                      PvuI
20901  TTCCCCTATC CGCTTATAGG CAAGACCGCA GTTGACAGCA TTACCCAGAA AAAGTTTCTT TGCGATCGCA CCCTTTGGCG CATCCCATTC TCCAGTAACT
       AAGGGGATAG GCGAATATCC GTTCTGGCGT CAACTGTCGT AATGGGTCTT TTTCAAAGAA ACGCTAGCGT GGGAAACCGC GTAGGGTAAG AGGTCATTGA
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Figure 15M

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                                                                                           BamHI
21001  TTATGTCCAT GGGCGCACTC ACAGACCTGG GCCAAAACCT TCTCTACGCC AACTCCGCCC ACGCGCTAGA CATGACTTTT GAGGTGGATC CCATGGACGA
       AATACAGGTA CCCGCGTGAG TGTCTGGACC CGGTTTTGGA AGAGATGCGG TTGAGGCGGG TGCGCGATCT GTACTGAAAA CTCCACCTAG GGTACCTGCT
21101  GCCCACCCTT CTTTATGTTT TGTTTGAAGT CTTTGACGTG GTCCGTGTGC ACCAGCCGCA CCGCGCCGTC ATCGAAACCG TGTACCTGCG CACGCCCTT
       CGGGTGGGAA GAAATACAAA ACAAACTTCA GAAACTGCAC CAGGCACACG TGGTCGGCGT GGCGCGGCAG TAGCTTTGGC ACATGGACGC GTGCGGGA
                                                                                                      BglII
21201  TCGGCCGGCA ACGCCACAAC ATAAAGAAGC AAGCAACATC AACAACAGCT GCCGCCATGG GCTCCAGTGA GCAGGAACTG AAAGCCATTG TCAAAGATCT
       AGCCGGCCGT TGCGGTGTTG TATTTCTTCG TTCGTTGTAG TTGTTGTCGA CGGCGGTACC CGAGGTCACT CGTCCTTGAC TTTCGGTAAC AGTTTCTAGA
21301  TGGTTGTGGG CCATATTTTT TGGGCACCTA TGACAAGCGC TTTCCAGGCT TTGTTTCTCC ACACAAGCTC GCCTGCGCCA TAGTCAATAC GGCCGGTC
       ACCAACACCC GGTATAAAAA ACCCGTGGAT ACTGTTCGCG AAAGGTCCGA AACAAAGAGG TGTGTTCGAG CGGACGCGGT ATCAGTTATG CCGGCCAG
21401  GAGACTGGGG GCGTACACTG GATGGCCTTT GCCTGGAACC CGCACTCAAA AACATGCTAC CTCTTTGAGC CCTTTGGCTT TTCTGACCAG CGACTCAAGC
       CTCTGACCCC CGCATGTGAC CTACCGGAAA CGGACCTTGG GCGTGAGTTT TTGTACGATG GAGAAACTCG GGAAACCGAA AAGACTGGTC GCTGAGTTCG
21501  AGGTTTACCA GTTTGAGTAC GAGTCACTCC TGCGCCGTAG CGCCATTGCT TCTTCCCCCG ACCGCTGTAT AACGCTGGAA AAGTCCACCC AAAGCGTACA
       TCCAAATGGT CAAACTCATG CTCAGTGAGG ACGCGGCATC GCGGTAACGA AGAAGGGGGC TGGCGACATA TTGCGACCTT TTCAGGTGGG TTTCGCATGT
21601  GGGGCCCAAC TCGGCCGCCT GTGGACTATT CTGCTGCATG TTTCTCCACG CCTTTGCCAA CTGGCCCCAA ACTCCCATGG ATCACAACCC CACCATGAA
       CCCCGGGTTG AGCCGGCGGA CACCTGATAA GACGACGTAC AAAGAGGTGC GGAAACGGTT GACCGGGGTT TGAGGGTACC TAGTGTTGGG GTGGTACTT
               KpnI
21701  CTTATTACCG GGGTACCCAA CTCCATGCTC AACAGTCCCC AGGTACAGCC CACCCTGCGT CGCAACCAGG AACAGCTCTA CAGCTTCCTG GAGCGCCAC
       GAATAATGGC CCCATGGGTT GAGGTACGAG TTGTCAGGGG TCCATGTCGG GTGGGACGCA GCGTTGGTCC TTGTCGAGAT GTCGAAGGAC CTCGCGGTG
21801  CGCCCTACTT CCGCAGCCAC AGTGCGCAGA TTAGGAGCGC CACTTCTTTT TGTCACTTGA AAAACATGTA AAAATAATGT ACTAGAGACA CTTTCAATAA
       GCGGGATGAA GGCGTCGGTG TCACGCGTCT AATCCTCGCG GTGAAGAAAA ACAGTGAACT TTTTGTACAT TTTTATTACA TGATCTCTGT GAAAGTTATT
21901  AGGCAAATGC TTTTATTTGT ACACTCTCGG GTGATTATTT ACCCCCACCC TTGCCGTCTG CGCCGTTTAA AAATCAAAGG GGTTCTGCCG CGCATCGCTA
       TCCGTTTACG AAAATAAACA TGTGAGAGCC CACTAATAAA TGGGGGTGGG AACGGCAGAC GCGGCAAATT TTTAGTTTCC CCAAGACGGC GCGTAGCGAT
22001  TGCGCCACTG GCAGGGACAC GTTGCGATAC TGGTGTTTAG TGCTCCACTT AAACTCAGGC ACAACCATCC GCGGCAGCTC GGTGAAGTTT TCACTCCACA
       ACGCGGTGAC CGTCCCTGTG CAACGCTATG ACCACAAATC ACGAGGTGAA TTTGAGTCCG TGTTGGTAGG CGCCGTCGAG CCACTTCAAA AGTGAGGTGT
                                                EcoRV
22101  GGCTGCGCAC CATCACCAAC GCGTTTAGCA GGTCGGGCGC CGATATCTTG AAGTCGCAGT TGGGGCCTCC GCCCTGCGCG CGCGAGTTGC GATACACAGG
       CCGACGCGTG GTAGTGGTTG CGCAAATCGT CCAGCCCGCG GCTATAGAAC TTCAGCGTCA ACCCCGGAGG CGGGACGCGC GCGCTCAACG CTATGTGTCC
22201  GTTGCAGCAC TGGAACACTA TCAGCGCCGG GTGGTGCACG CTGGCCAGCA CGCTCTTGTC GGAGATCAGA TCCGCGTCCA GGTCCTCCGC GTTGCTCAGG
       CAACGTCGTG ACCTTGTGAT AGTCGCGGCC CACCACGTGC GACCGGTCGT GCGAGAACAG CCTCTAGTCT AGGCGCAGGT CCAGGAGGCG CAACGAGTCC
22301  GCGAACGGAG TCAACTTTGG TAGCTGCCTT CCCAAAAAGG GCGCGTGCCC AGGCTTTGAG TTGCACTCGC ACCGTAGTGG CATCAAAAGG TGACCGTGCC
       CGCTTGCCTC AGTTGAAACC ATCGACGGAA GGGTTTTTCC CGCGCACGGG TCCGAAACTC AACGTGAGCG TGGCATCACC GTAGTTTTCC ACTGGCACGG
22401  CGGTCTGGGC GTTAGGATAC AGCGCCTGCA TAAAAGCCTT GATCTGCTTA AAAGCCACCT GAGCCTTTGC GCCTTCAGAG AAGAACATGC CGCAAGACTT
       GCCAGACCCG CAATCCTATG TCGCGGACGT ATTTTCGGAA CTAGACGAAT TTTCGGTGGA CTCGGAAACG CGGAAGTCTC TTCTTGTACG    TTCTGAA
                        SfiI                                          BglII
22501  GCCGGAAAAC TGATTGGCCG GACAGGCCGC GTCGTGCACG CAGCACCTTG CGTCGGTGTT GGAGATCTGC ACCACATTTC GGCCCCACCG GTTCTTCACG
       CGGCCTTTTG ACTAACCGGC CTGTCCGGCG CAGCACGTGC GTCGTGGAAC GCAGCCACAA CCTCTAGACG TGGTGTAAAG CCGGGGTGGC CAAGAAGTGC
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Figure 15N

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22601  ATCTTGGCCT TGCTAGACTG CTCCTTCAGC GCGCGCTGCC CGTTTTCGCT CGTCACATCC ATTTCAATCA CGTGCTCCTT ATTTATCATA ATGCTTCCGT
       TAGAACCGGA ACGATCTGAC GAGGAAGTCG CGCGCGACGG GCAAAAGCGA GCAGTGTAGG TAAAGTTAGT GCACGAGGAA TAAATAGTAT TACGAAGGCA
                                                                                                                PstI
22701  GTAGACACTT AAGCTCGCCT TCGATCTCAG CGCAGCGGTG CAGCCACAAC GCGCAGCCCG TGGGCTCGTG ATGCTTGTAG GTCACCTCTG CAAACGACTG
       CATCTGTGAA TTCGAGCGGA AGCTAGAGTC GCGTCGCCAC GTCGGTGTTG CGCGTCGGGC ACCCGAGCAC TACGAACATC CAGTGGAGAC GTTTGCTGAC
       PstI       PstI
22801  CAGGTACGCC TGCAGGAATC GCCCCATCAT CGTCACAAAG GTCTTGTTGC TGGTGAAGGT CAGCTGCAAC CCGCGGTGCT CCTCGTTCAG CCAGGTCTTG
       GTCCATGCGG ACGTCCTTAG CGGGGTAGTA GCAGTGTTTC CAGAACAACG ACCACTTCCA GTCGACGTTG GGCGCCACGA GGAGCAAGTC GGTCCAGAAC
22901  CATACGGCCG CCAGAGCTTC CACTTGGTCA GGCAGTAGTT TGAAGTTCGC CTTTAGATCG TTATCCACGT GGTACTTGTC CATCAGCGCG CGCGCAGCCT
       GTATGCCGGC GGTCTCGAAG GTGAACCAGT CCGTCATCAA ACTTCAAGCG GAAATCTAGC AATAGGTGCA CCATGAACAG GTAGTCGCGC GCGCGTCGGA
                               PvuI
23001  CCATGCCCTT CTCCCACGCA GACACGATCG GCACACTCAG CGGGTTCATC ACCGTAATTT CACTTTCCGC TTCGCTGGGC TCTTCCTCTT CCTCTTGCGT
       GGTACGGGAA GAGGGTGCGT CTGTGCTAGC CGTGTGAGTC GCCCAAGTAG TGGCATTAAA GTGAAAGGCG AAGCGACCCG AGAAGGAGAA GGAGAACGCA
23101  CCGCATACCA CGCGCCACTG GGTCGTCTTC ATTCAGCCGC CGCACTGTGC GCTTACCTCC TTTGCCATGC TTGATTAGCA CCGGTGGGTT GCTGAAACCC
       GGCGTATGGT GCGCGGTGAC CCAGCAGAAG TAAGTCGGCG GCGTGACACG CGAATGGAGG AAACGGTACG AACTAATCGT GGCCACCCAA CGACTTTGGG
23201  ACCATTTGTA GCGCCACATC TTCTCTTTCT TCCTCGCTGT CCACGATTAC CTCTGGTGAT GGCGGGCGCT CGGGCTTGGG AGAAGGGCGC TTCTTTTTCT
       TGGTAAACAT CGCGGTGTAG AAGAGAAAGA AGGAGCGACA GGTGCTAATG GAGACCACTA CCGCCCGCGA GCCCGAACCC TCTTCCCGCG AAGAAAAAGA
23301  TCTTGGGCGC AATGGCCAAA TCCGCCGCCG AGGTCGATGG CCGCGGGCTG GGTGTGCGCG GCACCAGCGC GTCTTGTGAT GAGTCTTCCT CGTCCTCGGA
       AGAACCCGCG TTACCGGTTT AGGCGGCGGC TCCAGCTACC GGCGCCCGAC CCACACGCGC CGTGGTCGCG CAGAACACTA CTCAGAAGGA GCAGGAGCCT
23401  CTCGATACGC CGCCTCATCC GCTTTTTTGG GGGCGCCCGG GGAGGCGGCG GCGACGGGGA CGGGGACGAC ACGTCCTCCA TGGTTGGGGG ACGTCGCGCC
       GAGCTATGCG GCGGAGTAGG CGAAAAAACC CCCGCGGGCC CCTCCGCCGC CGCTGCCCCT GCCCCTGCTG TGCAGGAGGT ACCAACCCCC TGCAGCGCG
23501  GCACCGCGTC CGCGCTCGGG GGTGGTTTCG CGCTGCTCCT CTTCCCGACT GGCCATTTCC TTCTCCTATA GGCAGAAAAA GATCATGGAG TCAGTCGAGA
       CGTGGCGCAG GCGCGAGCCC CCACCAAAGC GCGACGAGGA GAAGGGCTGA CCGGTAAAGG AAGAGGATAT CCGTCTTTTT CTAGTACCTC AGTCAGCTCT
23601  AGAAGGACAG CCTAACCGCC CCCTCTGAGT TCGCCACCAC CGCCTCCACC GATGCCGCCA ACGCGCCTAC CACCTTCCCC GTCGAGGCAC CCCCGCTTGA
       TCTTCCTGTC GGATTGGCGG GGGAGACTCA AGCGGTGGTG GCGGAGGTGG CTACGGCGGT TGCGCGGATG GTGGAAGGGG CAGCTCCGTG GGGGCGAACT
23701  GGAGGAGGAA GTGATTATCG AGCAGGACCC AGGTTTTGTA AGCGAAGACG ACGAGGACCG CTCAGTACCA ACAGAGGATA AAAAGCAAGA CCAGGACAAC
       CCTCCTCCTT CACTAATAGC TCGTCCTGGG TCCAAAACAT TCGCTTCTGC TGCTCCTGGC GAGTCATGGT TGTCTCCTAT TTTTCGTTCT GGTCCTGTTG
                                                                                                    PstI
23801  GCAGAGGCAA ACGAGGAACA AGTCGGGCGG GGGGACGAAA GGCATGGCGA CTACCTAGAT GTGGGAGACG ACGTGCTGTT GAAGCATCTG CAGCGCCAGT
       CGTCTCCGTT TGCTCCTTGT TCAGCCCGCC CCCCTGCTTT CCGTACCGCT GATGGATCTA CACCCTCTGC TGCACGACAA CTTCGTAGAC GTCGCGGTCA
23901  GCGCCATTAT CTGCGACGCG TTGCAAGAGC GCAGCGATGT GCCCCTCGCC ATAGCGGATG TCAGCCTTGC CTACGAACGC CACCTATTCT CACCGCGCGT
       CGCGGTAATA GACGCTGCGC AACGTTCTCG CGTCGCTACA CGGGGAGCGG TATCGCCTAC AGTCGGAACG GATGCTTGCG GTGGATAAGA GTGGCGCGCA
24001  ACCCCCCAAA CGCCAAGAAA ACGGCACATG CGAGCCCAAC CCGCGCCTCA ACTTCTACCC CGTATTTGCC GTGCCAGAGG TGCTTGCCAC CTATCACATC
       TGGGGGGTTT GCGGTTCTTT TGCCGTGTAC GCTCGGGTTG GGCGCGGAGT TGAAGATGGG GCATAAACGG CACGGTCTCC ACGAACGGTG GATAGTGTAG
                                                                                                           EcoRV
24101  TTTTTCCAAA ACTGCAAGAT ACCCCTATCC TGCCGTGCCA ACCGCAGCCG AGCGGACAAG CAGCTGGCCT TGCGGCAGGG CGCTGTCATA CCTGATATCG
       AAAAAGGTTT TGACGTTCTA TGGGGATAGG ACGGCACGGT TGGCGTCGGC TCGCCTGTTC GTCGACCGGA ACGCCGTCCC GCGACAGTAT GGACTATAGC
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Figure 15D

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24201 CCTCGCTCAA CGAAGTGCCA AAAATCTTTG AGGGTCTTGG ACGCGACGAG AAGCGCGCGG CAAACGCTCT GCAACAGGAA AACAGCGAAA ATGAAAGTCA
GGAGCGAGTT GCTTCACGGT TTTTAGAAAC TCCCAGAACC TGCGCTGCTC TTCGCGCGCC GTTTGCGAGA CGTTGTCCTT TTGTCGCTTT TACTTTCAGT

XhoI

24301 CTCTGGAGTG TTGGTGGAAC TCGAGGGTGA CAACGCGCGC CTAGCCGTAC TAAAACGCAG CATCGAGGTC ACCCACTTTG CCTACCCGGC ACTTAACCTA
GAGACCTCAC AACCACCTTG AGCTCCCACT GTTGCGCGCG GATCGGCATG ATTTTGCGTC GTAGCTCCAG TGGGTGAAAC GGATGGGCCG TGAATTGGAT

24401 CCCCCCAAGG TCATGAGCAC AGTCATGAGT GAGCTGATCG TGCGCCGTGC GCAGCCCCTG GAGAGGGATG CAAATTTGCA AGAACAAACA GAGGAGGGCC
GGGGGGTTCC AGTACTCGTG TCAGTACTCA CTCGACTAGC ACGCGGCACG CGTCGGGGAC CTCTCCCTAC GTTTAAACGT TCTTGTTTGT CTCCTCCCGG

24501 TACCCGCAGT TGGCGACGAG CAGCTAGCGC GCTGGCTTCA AACGCGCGAG CCTGCCGACT TGGAGGAGCG ACGCAAACTA ATGATGGCCG CAGTGCTCGT
ATGGGCGTCA ACCGCTGCTC GTCGATCGCG CGACCGAAGT TTGCGCGCTC GGACGGCTGA ACCTCCTCGC TGCGTTTGAT TACTACCGGC GTCACGAGCA

SphI

24601 TACCGTGGAG CTTGAGTGCA TGCAGCGGTT CTTTGCTGAC CCGGAGATGC AGCGCAAGCT AGAGGAAACA TTGCACTACA CCTTTCGACA GGGCTACGTA
ATGGCACCTC GAACTCACGT ACGTCGCCAA GAAACGACTG GGCCTCTACG TCGCGTTCGA TCTCCTTTGT AACGTGATGT GGAAAGCTGT CCCGATGCAT

BglII

24701 CGCCAGGCCT GCAAGATCTC CAACGTGGAG CTCTGCAACC TGGTCTCCTA CCTTGGAATT TTGCACGAAA ACCGCCTTGG GCAAAACGTG CTTCATTCCA
GCGGTCCGGA CGTTCTAGAG GTTGCACCTC GAGACGTTGG ACCAGAGGAT GGAACCTTAA AACGTGCTTT TGGCGGAACC CGTTTTGCAC GAAGTAAGGT

AscI

24801 CGCTCAAGGG CGAGGCGCGC CGCGACTACG TCCGCGACTG CGTTTACTTA TTTCTATGCT ACACCTGGCA GACGGCCATG GGCGTTTGGC AGCAGTGCTT
GCGAGTTCCC GCTCCGCGCG GCGCTGATGC AGGCGCTGAC GCAAATGAAT AAAGATACGA TGTGGACCGT CTGCCGGTAC CCGCAAACCG TCGTCACGAA

PstI

24901 GGAGGAGTGC AACCTCAAGG AGCTGCAGAA ACTGCTAAAG CAAAACTTGA AGGACCTATG GACGGCCTTC AACGAGCGCT CCGTGGCCGC GCACCTGGCG
CCTCCTCACG TTGGAGTTCC TCGACGTCTT TGACGATTTC GTTTTGAACT TCCTGGATAC CTGCCGGAAG TTGCTCGCGA GGCACCGGCG CGTGGACCGC

25001 GACATCATTT TCCCCGAACG CCTGCTTAAA ACCCTGCAAC AGGGTCTGCC AGACTTCACC AGTCAAAGCA TGTTGCAGAA CTTTAGGAAC TTTATCCTAG
CTGTAGTAAA AGGGGCTTGC GGACGAATTT TGGGACGTTG TCCCAGACGG TCTGAAGTGG TCAGTTTCGT ACAACGTCTT GAAATCCTTG AAATAGGATC

25101 AGCGCTCAGG AATCTTGCCC GCCACCTGCT GTGCACTTCC TAGCGACTTT GTGCCCATTA AGTACCGCGA ATGCCCTCCG CCGCTTTGGG GCCACTGCTA
TCGCGAGTCC TTAGAACGGG CGGTGGACGA CACGTGAAGG ATCGCTGAAA CACGGGTAAT TCATGGCGCT TACGGGAGGC GGCGAAACCC CGGTGACGAT

PstI

25201 CCTTCTGCAG CTAGCCAACT ACCTTGCCTA CCACTCTGAC ATAATGGAAG ACGTGAGCGG TGACGGTCTA CTGGAGTGTC ACTGTCGCTG CAACCTATGC
GGAAGACGTC GATCGGTTGA TGGAACGGAT GGTGAGACTG TATTACCTTC TGCACTCGCC ACTGCCAGAT GACCTCACAG TGACAGCGAC GTTGGATACG

KpnI PstI

25301 ACCCCGCACC GCTCCCTGGT TTGCAATTCG CAGCTGCTTA ACGAAAGTCA AATTATCGGT ACCTTTGAGC TGCAGGGTCC CTCGCCTGAC GAAAAGTCCG
TGGGGCGTGG CGAGGGACCA AACGTTAAGC GTCGACGAAT TGCTTTCAGT TTAATAGCCA TGGAAACTCG ACGTCCCAGG GAGCGGACTG CTTTTCAGGC

25401 CGGCTCCGGG GTTGAAACTC ACTCCGGGGC TGTGGACGTC GGCTTACCTT CGCAAATTTG TACCTGAGGA CTACCACGCC CACGAGATTA GGTTCTACGA
GCCGAGGCCC CAACTTTGAG TGAGGCCCCG ACACCTGCAG CCGAATGGAA GCGTTTAAAC ATGGACTCCT GATGGTGCGG GTGCTCTAAT CCAAGATGCT

25501 AGACCAATCC CGCCCGCCTA ATGCGGAGCT TACCGCCTGC GTCATTACCC AGGGCCACAT TCTTGGCCAA TTGCAAGCCA TCAACAAAGC CCGCCAAGAG
TCTGGTTAGG GCGGGCGGAT TACGCCTCGA ATGGCGGACG CAGTAATGGG TCCCGGTGTA AGAACCGGTT AACGTTCGGT AGTTGTTTCG GGCGGTTCTC

25601 TTTCTGCTAC GAAAGGGACG GGGGGTTTAC TTGGACCCCC AGTCCGGCGA GGAGCTCAAC CCAATCCCCC CGCCGCCGCA GCCCTATCAG CAGCAGCCGC
AAAGACGATG CTTTCCCTGC CCCCCAAATG AACCTGGGGG TCAGGCCGCT CCTCGAGTTG GGTTAGGGGG GCGGCGGCGT CGGGATAGTC GTCGTCGGCG

Figure 15P

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                                           PstI
25701  GGGCCCTTGC TTCCCAGGAT GGCACCCAAA AAGAAGCTGC AGCTGCCGCC GCTACCCACG GACGAGGAGG AATACTGGGA CAGTCAGGCA GAGGAGGTTT
       CCCGGGAACG AAGGGTCCTA CCGTGGGTTT TTCTTCGACG TCGACGGCGG CGATGGGTGC CTGCTCCTCC TTATGACCCT GTCAGTCCGT CTCCTCCAAA
                                                          HindIII
25801  TGGACGAGGA GGAGGAGGAC ATGATGGAAG ACTGGGAGAG CCTAGACGAG GAAGCTTCCG AGGTCGAAGA GGTGTCAGAC GAAACACCGT CACCCTCGGT
       ACCTGCTCCT CCTCCTCCTG TACTACCTTC TGACCCTCTC GGATCTGCTC CTTCGAAGGC TCCAGCTTCT CCACAGTCTG CTTTGTGGCA GTGGGAGCCA
25901  CGCATTCCCC TCGCCGGCGC CCCAGAAATC GGCAACCGGT TCCAGCATGG CTACAACCTC CGCTCCTCAG GCGCCGCCGG CACTGCCCGT TCGCCGACCC
       GCGTAAGGGG AGCGGCCGCG GGGTCTTTAG CCGTTGGCCA AGGTCGTACC GATGTTGGAG GCGAGGAGTC CGCGGCGGCC GTGACGGGCA AGCGGCTGGG
26001  AACCGTAGAT GGGACACCAC TGGAACCAGG GCCGGTAAGT CCAAGCAGCC GCCGCCGTTA GCCCAAGAGC AACAACAGCG CCAAGGCTAC CGCTCATGGC
       TTGGCATCTA CCCTGTGGTG ACCTTGGTCC CGGCCATTCA GGTTCGTCGG CGGCGGCAAT CGGGTTCTCG TTGTTGTCGC GGTTCCGATG GCGAGTACCG
26101  GCGGGCACAA GAACGCCATA GTTGCTTGCT TGCAAGACTG TGGGGGCAAC ATCTCCTTCG CCCGCCGCTT TCTTCTCTAC CATCACGGCG TGGCTTCCC
       CGCCCGTGTT CTTGCGGTAT CAACGAACGA ACGTTCTGAC ACCCCCGTTG TAGAGGAAGC GGGCGGCGAA AGAAGAGATG GTAGTGCCGC ACCGAAGGG
26201  CCGTAACATC CTGCATTACT ACCGTCATCT CTACAGCCCA TACTGCACCG GCGGCAGCGG CAGCAACAGC AGCGGCCACA CAGAAGCAAA GGCGACCGGA
       GGCATTGTAG GACGTAATGA TGGCAGTAGA GATGTCGGGT ATGACGTGGC CGCCGTCGCC GTCGTTGTCG TCGCCGGTGT GTCTTCGTTT CCGCTGGCCT
26301  TAGCAAGACT CTGACAAAGC CCAAGAAATC CACAGCGGCG GCAGCAGCAG GAGGAGGAGC GCTGCGTCTG GCGCCCAACG AACCCGTATC GACCCGCGAG
       ATCGTTCTGA GACTGTTTCG GGTTCTTTAG GTGTCGCCGC CGTCGTCGTC CTCCTCCTCG CGACGCAGAC CGCGGGTTGC TTGGGCATAG CTGGGCGCTC
26401  CTTAGAAACA GGATTTTTCC CACTCTGTAT GCTATATTTC AACAGAGCAG GGGCCAAGAA CAAGAGCTGA AAATAAAAAA CAGGTCTCTG CGATCCCTCA
       GAATCTTTGT CCTAAAAAGG GTGAGACATA CGATATAAAG TTGTCTCGTC CCCGGTTCTT GTTCTCGACT TTTATTTTTT GTCCAGAGAC GCTAGGGAGT
26501  CCCGCAGCTG CCTGTATCAC AAAAGCGAAG ATCAGCTTCG GCGCACGCTG GAAGACGCGG AGGCTCTCTT CAGTAAATAC TGCGCGCTGA CTCTTAAGGA
       GGGCGTCGAC GGACATAGTG TTTTCGCTTC TAGTCGAAGC CGCGTGCGAC CTTCTGCGCC TCCGAGAGAA GTCATTTATG ACGCGCGACT GAGAATTCCT
26601  CTAGTTTCGC GCCCTTTCTC AAATTTAAGC GCGAAAACTA CGTCATCTCC AGCGGCCACA CCCGGCGCCA GCACCTGTTG TCAGCGCCAT TATGAGCAAG
       GATCAAAGCG CGGGAAAGAG TTTAAATTCG CGCTTTTGAT GCAGTAGAGG TCGCCGGTGT GGGCCGCGGT CGTGGACAAC AGTCGCGGTA ATACTCGTTC
26701  GAAATTCCCA CCCCCTACAT GTGGAGTTAC CAGCCACAAA TGGGACTTGC GGCTGGAGCT GCCCAAGACT ACTCAACCCG AATAAACTAC ATGAGCGCGG
       CTTTAAGGGT GGGGGATGTA CACCTCAATG GTCGGTGTTT ACCCTGAACG CCGACCTCGA CGGGTTCTGA TGAGTTGGGC TTATTTGATG TACTCGCGCC
                  EcoRV                                      EcoRI
26801  GACCCCACAT GATATCCCGG GTCAACGGAA TACGCGCCCA CCGAAACCGA ATTCTCCTGG AACAGGCGGC TATTACCACC ACACCTCGTA ATAACCTTAA
       CTGGGGTGTA CTATAGGGCC CAGTTGCCTT ATGCGCGGGT GGCTTTGGCT TAAGAGGACC TTGTCCGCCG ATAATGGTGG TGTGGAGCAT TATTGGAATT
26901  TCCCCGTAGT TGGCCCGCTG CCCTGGTGTA CCAGGAAAGT CCCGCTCCCA CCACTGTGGT ACTTCCCAGA GACGCCCAGG CCGAAGTTCA GATGACTAAC
       AGGGGCATCA ACCGGGCGAC GGGACCACAT GGTCCTTTCA GGGCGAGGGT GGTGACACCA TGAAGGGTCT CTGCGGGTCC GGCTTCAAGT CTACTGATTG
27001  TCAGGGGCGC AGCTTGCGGG CGGCTTTCGT CACAGGGTGC GGTCGCCCGG GCAGGGTATA ACTCACCTGA CAATCAGAGG GCGAGGTATT CAGCTCAACG
       AGTCCCCGCG TCGAACGCCC GCCGAAAGCA GTGTCCCACG CCAGCGGGCC CGTCCCATAT TGAGTGGACT GTTAGTCTCC CGCTCCATAA GTCGAGTTGC
27101  ACGAGTCGGT GAGCTCCTCG CTTGGTCTCC GTCCGGACGG GACATTTCAG ATCGGCGGCG CCGGCCGCTC TTCATTCACG CCTCGTCAGG CAATCCTAAC
       TGCTCAGCCA CTCGAGGAGC GAACCAGAGG CAGGCCTGCC CTGTAAAGTC TAGCCGCCGC GGCCGGCGAG AAGTAAGTGC GGAGCAGTCC GTTAGGATTG
        PstI
27201  TCTGCAGACC TCGTCCTCTG AGCCGCGCTC TGGAGGCATT GGAACTCTGC AATTTATTGA GGAGTTTGTG CCATCGGTCT ACTTTAACCC CTTCTCGGGA
       AGACGTCTGG AGCAGGAGAC TCGGCGCGAG ACCTCCGTAA CCTTGAGACG TTAAATAACT CCTCAAACAC GGTAGCCAGA TGAAATTGGG GAAGAGCCCT
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Figure 15Q

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27301  CCTCCCGGCC ACTATCCGGA TCAATTTATT CCTAACTTTG ACGCGGTAAA GGACTCGGCG GACGGCTACG ACTGAATGTT AAGTGGAGAG GCAGAGCAAC
       GGAGGGCCGG TGATAGGCCT AGTTAAATAA GGATTGAAAC TGCGCCATTT CCTGAGCCGC CTGCCGATGC TGACTTACAA TTCACCTCTC CGTCTCGTTG
27401  TGCGCCTGAA ACACCTGGTC CACTGTCGCC GCCACAAGTG CTTTGCCCGC GACTCCGGTG AGTTTTGCTA CTTTGAATTG CCCGAGGATC ATATCGAGGG
       ACGCGGACTT TGTGGACCAG GTGACAGCGG CGGTGTTCAC GAAACGGGCG CTGAGGCCAC TCAAAACGAT GAAACTTAAC GGGCTCCTAG TATAGCTCCC
27501  CCCGGCGCAC GGCGTCCGGC TTACCGCCCA GGGAGAGCTT GCCCGTAGCC TGATTCGGGA GTTTACCCAG CGCCCCCTGC TAGTTGAGCG GGACAGGGGA
       GGGCCGCGTG CCGCAGGCCG AATGGCGGGT CCCTCTCGAA CGGGCATCGG ACTAAGCCCT CAAATGGGTC GCGGGGGACG ATCAACTCGC CCTGTCCCCT
                                                         BglII
27601  CCCTGTGTTC TCACTGTGAT TTGCAACTGT CCTAACCCTG GATTACATCA AGATCTTTGT TGCCATCTCT GTGCTGAGTA TAATAAATAC AGAAATTAAA
       GGGACACAAG AGTGACACTA AACGTTGACA GGATTGGGAC CTAATGTAGT TCTAGAAACA ACGGTAGAGA CACGACTCAT ATTATTTATG TCTTTAATTT
27701  ATATACTGGG GCTCCTATCG CCATCCTGTA AACGCCACCG TCTTCACCCG CCCAAGCAAA CCAAGGCGAA CCTTACCTGG TACTTTTAAC ATCTCTCCCT
       TATATGACCC CGAGGATAGC GGTAGGACAT TTGCGGTGGC AGAAGTGGGC GGGTTCGTTT GGTTCCGCTT GGAATGGACC ATGAAAATTG TAGAGAGGGA
27801  CTGTGATTTA CAACAGTTTC AACCCAGACG GAGTGAGTCT ACGAGAGAAC CTCTCCGAGC TCAGCTACTC CATCAGAAAA AACACCACCC TCCTTACCTG
       GACACTAAAT GTTGTCAAAG TTGGGTCTGC CTCACTCAGA TGCTCTCTTG GAGAGGCTCG AGTCGATGAG GTAGTCTTTT TTGTGGTGGG AGGAATGGAC
27901  CCGGGAACGT ACGAGTGCGT CACCGGCCGC TGCACCACAC CTACCGCCTG ACCGTAAACC AGACTTTTTC CGGACAGACC TCAATAACTC TGTTTACCAG
       GGCCCTTGCA TGCTCACGCA GTGGCCGGCG ACGTGGTGTG GATGGCGGAC TGGCATTTGG TCTGAAAAAG GCCTGTCTGG AGTTATTGAG ACAAATGGTC
28001  AACAGGAGGT GAGCTTAGAA AACCCTTAGG GTATTAGGCC AAAGGCGCAG CTACTGTGGG GTTTATGAAC AATTCAAGCA ACTCTACGGG CTATTCTAAT
       TTGTCCTCCA CTCGAATCTT TTGGGAATCC CATAATCCGG TTTCCGCGTC GATGACACCC CAAATACTTG TTAAGTTCGT TGAGATGCCC GATAAGATTA
             XbaI
28101  TCAGGTTTCT CTAGAATCGG GGTTGGGGTT ATTCTCTGTC TTGTGATTCT CTTTATTCTT ATACTAACGC TTCTCTGCCT AAGGCTCGCC GCCTGCTGTG
       AGTCCAAAGA GATCTTAGCC CCAACCCCAA TAAGAGACAG AACACTAAGA GAAATAAGAA TATGATTGCG AAGAGACGGA TTCCGAGCGG CGGACGACAC
28201  TGCACATTTG CATTTATTGT CAGCTTTTTA AACGCTGGGG TCGCCACCCA AGATGATTAG GTACATAATC CTAGGTTTAC TCACCCTTGC GTCAGCCCAC
       ACGTGTAAAC GTAAATAACA GTCGAAAAAT TTGCGACCCC AGCGGTGGGT TCTACTAATC CATGTATTAG GATCCAAATG AGTGGGAACG CAGTCGGGTG
        KpnI
28301  GGTACCACCC AAAAGGTGGA TTTTAAGGAG CCAGCCTGTA ATGTTACATT CGCAGCTGAA GCTAATGAGT GCACCACTCT TATAAAATGC ACCACAGAAC
       CCATGGTGGG TTTTCCACCT AAAATTCCTC GGTCGGACAT TACAATGTAA GCGTCGACTT CGATTACTCA CGTGGTGAGA ATATTTTACG TGGTGTCTTG
28401  ATGAAAAGCT GCTTATTCGC CACAAAAACA AAATTGGCAA GTATGCTGTT TATGCTATTT GGCAGCCAGG TGACACTACA GAGTATAATG TTACAGTTTT
       TACTTTTCGA CGAATAAGCG GTGTTTTTGT TTTAACCGTT CATACGACAA ATACGATAAA CCGTCGGTCC ACTGTGATGT CTCATATTAC AATGTCAAAA
                              Bst1107I
28501  CCAGGGTAAA AGTCATAAAA CTTTTATGTA TACTTTTCCA TTTTATGAAA TGTGCGACAT TACCATGTAC ATGAGCAAAC AGTATAAGTT GTGGCCCCCA
       GGTCCCATTT TCAGTATTTT GAAAATACAT ATGAAAAGGT AAAATACTTT ACACGCTGTA ATGGTACATG TACTCGTTTG TCATATTCAA CACCGGGGGT
28601  CAAAATTGTG TGGAAAACAC TGGCACTTTC TGCTGCACTG CTATGCTAAT TACAGTGCTC GCTTTGGTCT GTACCCTACT CTATATTAAA TACAAAAGCA
       GTTTTAACAC ACCTTTTGTG ACCGTGAAAG ACGACGTGAC GATACGATTA ATGTCACGAG CGAAACCAGA CATGGGATGA GATATAATTT ATGTTTTCGT
28701  GACGCAGCTT TATTGAGGAA AAGAAAATGC CTTAATTTAC TAAGTTACAA AGCTAATGTC ACCACTAACT GCTTTACTCG CTGCTTGCAA AACAAATTCA
       CTGCGTCGAA ATAACTCCTT TTCTTTTACG GAATTAAATG ATTCAATGTT TCGATTACAG TGGTGATTGA CGAAATGAGC GACGAACGTT TTGTTTAAGT
28801  AAAAGTTAGC ATTATAATTA GAATAGGATT TAAACCCCCC GGTCATTTCC TGCTCAATAC CATTCCCCTG AACAATTGAC TCTATGTGGG ATATGCTCCA
       TTTTCAATCG TAATATTAAT CTTATCCTAA ATTTGGGGGG CCAGTAAAGG ACGAGTTATG GTAAGGGGAC TTGTTAACTG AGATACACCC TATACGAGGT
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Figure 15R

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28901  GCGCTACAAC CTTGAAGTCA GGCTTCCTGG ATGTCAGCAT CTGACTTTGG CCAGCACCTG TCCCGCGGAT TTGTTCCAGT CCAACTACAG CGACCCACCC
       CGCGATGTTG GAACTTCAGT CCGAAGGACC TACAGTCGTA GACTGAAACC GGTCGTGGAC AGGGCGCCTA AACAAGGTCA GGTTGATGTC GCTGGGTGGG
29001  TAACAGAGAT GACCAACACA ACCAACGCGG CCGCCGCTAC CGGACTTACA TCTACCACAA ATACACCCCA AGTTTCTGCC TTTGTCAATA ACTGGGATAA
       ATTGTCTCTA CTGGTTGTGT TGGTTGCGCC GGCGGCGATG GCCTGAATGT AGATGGTGTT TATGTGGGGT TCAAAGACGG AAACAGTTAT TGACCCTATT
29101  CTTGGGCATG TGGTGGTTCT CCATAGCGCT TATGTTTGTA TGCCTTATTA TTATGTGGCT CATCTGCTGC CTAAAGCGCA AACGCGCCCG ACCACCCATC
       GAACCCGTAC ACCACCAAGA GGTATCGCGA ATACAAACAT ACGGAATAAT AATACACCGA GTAGACGACG GATTTCGCGT TTGCGCGGGC TGGTGGGTAG
29201  TATAGTCCCA TCATTGTGCT ACACCCAAAC AATGATGGAA TCCATAGATT GGACGGACTG AAACACATGT TCTTTTCTCT TACAGTATGA TTAAATGAGA
       ATATCAGGGT AGTAACACGA TGTGGGTTTG TTACTACCTT AGGTATCTAA CCTGCCTGAC TTTGTGTACA AGAAAAGAGA ATGTCATACT AATTTACTCT
                 XhoI
29301  CATGATTCCT CGAGTTTTTA TATTACTGAC CCTTGTTGCG CTTTTTTGTG CGTGCTCCAC ATTGGCTGCG GTTTCTCACA TCGAAGTAGA CTGCATTCTA
       GTACTAAGGA GCTCAAAAAT ATAATGACTG GGAACAACGC GAAAAAACAC GCACGAGGTG TAACCGACGC CAAAGAGTGT AGCTTCATCT GACGTAAGAT
                                                    PstI
29401  GCCTTCACAG TCTATTTGCT TTACGGATTT GTCACCCTCA CGCTCATCTG CAGCCTCATC ACTGTGGTCA TCGCCTTTAT CCAGTGCATT GACTGGGTCT
       CGGAAGTGTC AGATAAACGA AATGCCTAAA CAGTGGGAGT GCGAGTAGAC GTCGGAGTAG TGACACCAGT AGCGGAAATA GGTCACGTAA CTGACCCAGA
                                                                      EcoRI
29501  GTGTGCGCTT TGCATATCTC AGACACCATC CCCAGTACAG GGACAGGACT ATAGCTGAGC TTCTTAGAAT TCTTTAATTA TGAAATTTAC TGTGACTTTT
       CACACGCGAA ACGTATAGAG TCTGTGGTAG GGGTCATGTC CCTGTCCTGA TATCGACTCG AAGAATCTTA AGAAATTAAT ACTTTAAATG ACACTGAAAA
29601  CTGCTGATTA TTTGCACCCT ATCTGCGTTT TGTTCCCCGA CCTCCAAGCC TCAAAGACAT ATATCATGCA GATTCACTCG TATATGGAAT ATTCCAAGTT
       GACGACTAAT AAACGTGGGA TAGACGCAAA ACAAGGGGCT GGAGGTTCGG AGTTTCTGTA TATAGTACGT CTAAGTGAGC ATATACCTTA TAAGGTTCAA
                                                                 PstI
29701  GCTACAATGA AAAAAGCGAT CTTTCCGAAG CCTGGTTATA TGCAATCATC TCTGTTATGG TGTTCTGCAG TACCATCTTA GCCCTAGCTA TATATCCCTA
       CGATGTTACT TTTTTCGCTA GAAAGGCTTC GGACCAATAT ACGTTAGTAG AGACAATACC ACAAGACGTC ATGGTAGAAT CGGGATCGAT ATATAGGGAT
29801  CCTTGACATT GGCTGGAACG CAATAGATGC CATGAACCAC CCAACTTTCC CCGCGCCCGC TATGCTTCCA CTGCAACAAG TTGTTGCCGG CGGCTTTGTC
       GGAACTGTAA CCGACCTTGC GTTATCTACG GTACTTGGTG GGTTGAAAGG GGCGCGGGCG ATACGAAGGT GACGTTGTTC AACAACGGCC GCCGAAACAG
                                                                                               XbaI
                                                                                           BglII
29901  CCAGCCAATC AGCCTCGCCC ACCTTCTCCC ACCCCCACTG AAATCAGCTA CTTTAATCTA ACAGGAGGAG ATGACTGACA CCCTAGATCT AGAAATGGAC
       GGTCGGTTAG TCGGAGCGGG TGGAAGAGGG TGGGGGTGAC TTTAGTCGAT GAAATTAGAT TGTCCTCCTC TACTGACTGT GGGATCTAGA TCTTTACCTG
30001  GGAATTATTA CAGAGCAGCG CCTGCTAGAA AGACGCAGGG CAGCGGCCGA GCAACAGCGC ATGAATCAAG AGCTCCAAGA CATGGTTAAC TTGCACCAGT
       CCTTAATAAT GTCTCGTCGC GGACGATCTT TCTGCGTCCC GTCGCCGGCT CGTTGTCGCG TACTTAGTTC TCGAGGTTCT GTACCAATTG AACGTGGTCA
30101  GCAAAAGGGG TATCTTTTGT CTCGTAAAGC AGGCCAAAGT CACCTACGAC AGTAATACCA CCGGACACCG CCTTAGCTAC AAGTTGCCAA CCAAGCGTTA
       CGTTTTCCCC ATAGAAAACA GAGCATTTCG TCCGGTTTCA GTGGATGCTG TCATTATGGT GGCCTGTGGC GGAATCGATG TTCAACGGTT GGTTCGCAAT
30201  GAAATTGGTG GTCATGGTGG GAGAAAAGCC CATTACCATA ACTCAGCACT CGGTAGAAAC CGAAGGCTGC ATTCACTCAC CTTGTCAAGG ACCTGAGGAT
       CTTTAACCAC CAGTACCACC CTCTTTTCGG GTAATGGTAT TGAGTCGTGA GCCATCTTTG GCTTCCGACG TAAGTGAGTG GAACAGTTCC TGGACTCCTA
                                        BglII
30301  CTCTGCACCC TTATTAAGAC CCTGTGCGGT CTCAAAGATC TTATTCCCTT TAACTAATAA AAAAAAATAA TAAAGCATCA CTTACTTAAA ATCAGTTAGC
       GAGACGTGGG AATAATTCTG GGACACGCCA GAGTTTCTAG AATAAGGGAA ATTGATTATT TTTTTTTATT ATTTCGTAGT GAATGAATTT TAGTCAATCG
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Figure 15S

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30401  AAATTTCTGT CCAGTTTATT CAGCAGCACC TCCTTGCCCT CCTCCCAGCT CTGGTATTGC AGCTTCCTCC TGGCTGCAAA CTTTCTCCAC AATCTAAATG
       TTTAAAGACA GGTCAAATAA GTCGTCGTGG AGGAACGGGA GGAGGGTCGA GACCATAACG TCGAAGGAGG ACCGACGTTT GAAAGAGGTG TTAGATTTAC
30501  GAATGTCAGT TTCCTCCTGT TCCTGTCCAT CCGCACCCAC TATCTTCATG TTGTTGCAGA TGAAGCGCGC AAGACCGTCT GAAGATACCT TCAACCCCGT
       CTTACAGTCA AAGGAGGACA AGGACAGGTA GGCGTGGGTG ATAGAAGTAC AACAACGTCT ACTTCGCGCG TTCTGGCAGA CTTCTATGGA AGTTGGGGCA
30601  GTATCCATAT GACACGGAAA CCGGTCCTCC AACTGTGCCT TTTCTTACTC CTCCCTTTGT ATCCCCCAAT GGGTTTCAAG AGAGTCCCCC TGGGGTACTC
       CATAGGTATA CTGTGCCTTT GGCCAGGAGG TTGACACGGA AAAGAATGAG GAGGGAAACA TAGGGGGTTA CCCAAAGTTC TCTCAGGGGG ACCCCATGAG
                                                 SphI
30701  TCTTTGCGCC TATCCGAACC TCTAGTTACC TCCAATGGCA TGCTTGCGCT CAAAATGGGC AACGGCCTCT CTCTGGACGA GGCCGGCAAC CTTACCTCCC
       AGAAACGCGG ATAGGCTTGG AGATCAATGG AGGTTACCGT ACGAACGCGA GTTTTACCCG TTGCCGGAGA GAGACCTGCT CCGGCCGTTG GAATGGAGGG
30801  AAAATGTAAC CACTGTGAGC CCACCTCTCA AAAAAACCAA GTCAAACATA AACCTGGAAA TATCTGCACC CCTCACAGTT ACCTCAGAAG CCCTAACTGT
       TTTTACATTG GTGACACTCG GGTGGAGAGT TTTTTTGGTT CAGTTTGTAT TTGGACCTTT ATAGACGTGG GGAGTGTCAA TGGAGTCTTC GGGATTGACA
30901  GGCTGCCGCC GCACCTCTAA TGGTCGCGGG CAACACACTC ACCATGCAAT CACAGGCCCC GCTAACCGTG CACGACTCCA AACTTAGCAT TGCCACCCAA
       CCGACGGCGG CGTGGAGATT ACCAGCGCCC GTTGTGTGAG TGGTACGTTA GTGTCCGGGG CGATTGGCAC GTGCTGAGGT TTGAATCGTA ACGGTGGGTT
31001  GGACCCCTCA CAGTGTCAGA AGGAAAGCTA GCCCTGCAAA CATCAGGCCC CCTCACCACC ACCGATAGCA GTACCCTTAC TATCACTGCC TCACCCCCTT
       CCTGGGGAGT GTCACAGTCT TCCTTTCGAT CGGGACGTTT GTAGTCCGGG GGAGTGGTGG TGGCTATCGT CATGGGAATG ATAGTGACGG AGTGGGGGAA
31101  TAACTACTGC CACTGGTAGC TTGGGCATTG ACTTGAAAGA GCCCATTTAT ACACAAAATG GAAAACTAGG ACTAAAGTAC GGGGCTCCTT TGCATGTAAC
       ATTGATGACG GTGACCATCG AACCCGTAAC TGAACTTTCT CGGGTAAATA TGTGTTTTAC CTTTTGATCC TGATTTCATG CCCCGAGGAA ACGTACATTG
31201  AGACGACCTA AACACTTTGA CCGTAGCAAC TGGTCCAGGT GTGACTATTA ATAATACTTC CTTGCAAACT AAAGTTACTG GAGCCTTGGG TTTTGATTCA
       TCTGCTGGAT TTGTGAAACT GGCATCGTTG ACCAGGTCCA CACTGATAAT TATTATGAAG GAACGTTTGA TTTCAATGAC CTCGGAACCC AAAACTAAGT
31301  CAAGGCAATA TGCAACTTAA TGTAGCAGGA GGACTAAGGA TTGATTCTCA AAACAGACGC CTTATACTTG ATGTTAGTTA TCCGTTTGAT GCTCAAAACC
       GTTCCGTTAT ACGTTGAATT ACATCGTCCT CCTGATTCCT AACTAAGAGT TTTGTCTGCG GAATATGAAC TACAATCAAT AGGCAAACTA CGAGTTTTGG
31401  AACTAAATCT AAGACTAGGA CAGGGCCCTC TTTTTATAAA CTCAGCCCAC AACTTGGATA TTAACTACAA CAAAGGCCTT TACTTGTTTA CAGCTTCAAT
       TTGATTTAGA TTCTGATCCT GTCCCGGGAG AAAAATATTT GAGTCGGGTG TTGAACCTAT AATTGATGTT GTTTCCGGAA ATGAACAAAT GTCGAAGTTA
                 HindIII
31501  CAATTCCAAA AAGCTTGAGG TTAACCTAAG CACTGCCAAG GGGTTGATGT TTGACGCTAC AGCCATAGCC ATTAATGCAG GAGATGGGCT TGAATTTGGT
       GTTAAGGTTT TTCGAACTCC AATTGGATTC GTGACGGTTC CCCAACTACA AACTGCGATG TCGGTATCGG TAATTACGTC CTCTACCCGA ACTTAAACCA
31601  TCACCTAATG CACCAAACAC AAATCCCCTC AAAACAAAAA TTGGCCATGG CCTAGAATTT GATTCAAACA AGGCTATGGT TCCTAAACTA GGAACTGGCC
       AGTGGATTAC GTGGTTTGTG TTTAGGGGAG TTTTGTTTTT AACCGGTACC GGATCTTAAA CTAAGTTTGT TCCGATACCA AGGATTTGAT CCTTGACCGG
31701  TTAGTTTTGA CAGCACAGGT GCCATTACAG TAGGAAACAA AAATAATGAT AAGCTAACTT TGTGGACCAC ACCAGCTCCA TCTCCTAACT GTAGACTAAA
       AATCAAAACT GTCGTGTCCA CGGTAATGTC ATCCTTTGTT TTTATTACTA TTCGATTGAA ACACCTGGTG TGGTCGAGGT AGAGGATTGA CATCTGATTT
31801  TGCAGAGAAA GATGCTAAAC TCACTTTGGT CTTAACAAAA TGTGGCAGTC AAATACTTGC TACAGTTTCA GTTTTGGCTG TTAAAGGCAG TTTGGCTCCA
       ACGTCTCTTT CTACGATTTG AGTGAAACCA GAATTGTTTT ACACCGTCAG TTTATGAACG ATGTCAAAGT CAAAACCGAC AATTTCCGTC AAACCGAGGT
31901  ATATCTGGAA CAGTTCAAAG TGCTCATCTT ATTATAAGAT TTGACGAAAA TGGAGTGCTA CTAAACAATT CCTTCCTGGA CCCAGAATAT TGGAACTTTA
       TATAGACCTT GTCAAGTTTC ACGAGTAGAA TAATATTCTA AACTGCTTTT ACCTCACGAT GATTTGTTAA GGAAGGACCT GGGTCTTATA ACCTTGAAAT
                BglII
32001  GAAATGGAGA TCTTACTGAA GGCACAGCCT ATACAAACGC TGTTGGATTT ATGCCTAACC TATCAGCTTA TCCAAAATCT CACGGTAAAA CTGCCAAAAG
       CTTTACCTCT AGAATGACTT CCGTGTCGGA TATGTTTGCG ACAACCTAAA TACGGATTGG ATAGTCGAAT AGGTTTTAGA GTGCCATTTT GACGGTTTTC
```

Figure 15T

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32101  TAACATTGTC AGTCAAGTTT ACTTAAACGG AGACAAAACT AAACCTGTAA CACTAACCAT TACACTAAAC GGTACACAGG AAACAGGAGA CACAACTCCA
       ATTGTAACAG TCAGTTCAAA TGAATTTGCC TCTGTTTTGA TTTGGACATT GTGATTGGTA ATGTGATTTG CCATGTGTCC TTTGTCCTCT GTGTTGAGGT
32201  AGTGCATACT CTATGTCATT TTCATGGGAC TGGTCTGGCC ACAACTACAT TAATGAAATA TTTGCCACAT CCTCTTACAC TTTTTCATAC ATTGCCCAA
       TCACGTATGA GATACAGTAA AAGTACCCTG ACCAGACCGG TGTTGATGTA ATTACTTTAT AAACGGTGTA GGAGAATGTG AAAAAGTATG TAACGGGTT
32301  AATAAAGAAT CGTTTGTGTT ATGTTTCAAC GTGTTTATTT TTCAATTGCA GAAAATTTCA AGTCATTTTT CATTCAGTAG TATAGCCCCA CCACCACATA
       TTATTTCTTA GCAAACACAA TACAAAGTTG CACAAATAAA AAGTTAACGT CTTTTAAAGT TCAGTAAAAA GTAAGTCATC ATATCGGGGT GGTGGTGTAT
32401  GCTTATACAG ATCACCGTAC CTTAATCAAA CTCACAGAAC CCTAGTATTC AACCTGCCAC CTCCCTCCCA ACACACAGAG TACACAGTCC TTTCTCCC
       CGAATATGTC TAGTGGCATG GAATTAGTTT GAGTGTCTTG GGATCATAAG TTGGACGGTG GAGGGAGGGT TGTGTGTCTC ATGTGTCAGG AAAGAGGG
32501  GCTGGCCTTA AAAAGCATCA TATCATGGGT AACAGACATA TTCTTAGGTG TTATATTCCA CACGGTTTCC TGTCGAGGCA AACGCTCATC AGTGATATT
       CGACCGGAAT TTTTCGTAGT ATAGTACCCA TTGTCTGTAT AAGAATCCAC AATATAAGGT GTGCCAAAGG ACAGCTCCGT TTGCGAGTAG TCACTATAA
32601  ATAAACTCCC CGGGCAGCTC ACTTAAGTTC ATGTCGCTGT CCAGCTGCTG AGCCACAGGC TGCTGTCCAA CTTGCGGTTG CTTAACGGGC GGCGAAGGA
       TATTTGAGGG GCCCGTCGAG TGAATTCAAG TACAGCGACA GGTCGACGAC TCGGTGTCCG ACGACAGGTT GAACGCCAAC GAATTGCCCG CCGCTTCCT
                                                                   PstI
32701  AAGTCCACGC CTACATGGGG GTAGAGTCAT AATCGTGCAT CAGGATAGGG CGGTGGTGCT GCAGCAGCGC GCGAATAAAC TGCTGCCGCC GCCGCTCCGT
       TTCAGGTGCG GATGTACCCC CATCTCAGTA TTAGCACGTA GTCCTATCCC GCCACCACGA CGTCGTCGCG CGCTTATTTG ACGACGGCGG CGGCGAGGCA
        PstI
32801  CCTGCAGGAA TACAACATGG CAGTGGTCTC CTCAGCGATG ATTCGCACCG CCCGCAGCAT AAGGCGCCTT GTCCTCCGGG CACAGCAGCG CACCCTGAT
       GGACGTCCTT ATGTTGTACC GTCACCAGAG GAGTCGCTAC TAAGCGTGGC GGGCGTCGTA TTCCGCGGAA CAGGAGGCCC GTGTCGTCGC GTGGGACTA
                              PstI
32901  TCACTTAAAT CAGCACAGTA ACTGCAGCAC AGCACCACAA TATTGTTCAA AATCCCACAG TGCAAGGCGC TGTATCCAAA GCTCATGGCG GGGACCACAG
       AGTGAATTTA GTCGTGTCAT TGACGTCGTG TCGTGGTGTT ATAACAAGTT TTAGGGTGTC ACGTTCCGCG ACATAGGTTT CGAGTACCGC CCCTGGTGTC
33001  AACCCACGTG GCCATCATAC CACAAGCGCA GGTAGATTAA GTGGCGACCC CTCATAAACA CGCTGGACAT AAACATTACC TCTTTTGGCA TGTTGTAATT
       TTGGGTGCAC CGGTAGTATG GTGTTCGCGT CCATCTAATT CACCGCTGGG GAGTATTTGT GCGACCTGTA TTTGTAATGG AGAAAACCGT ACAACATTAA
                   KpnI                                                                                     PstI
33101  CACCACCTCC CGGTACCATA TAAACCTCTG ATTAAACATG GCGCCATCCA CCACCATCCT AAACCAGCTG GCCAAAACCT GCCCGCCGGC TATACACTG
       GTGGTGGAGG GCCATGGTAT ATTTGGAGAC TAATTTGTAC CGCGGTAGGT GGTGGTAGGA TTTGGTCGAC CGGTTTTGGA CGGGCGGCCG ATATGTGAC
       PstI                                                                          EcoRV
33201  AGGGAACCGG GACTGGAACA ATGACAGTGG AGAGCCCAGG ACTCGTAACC ATGGATCATC ATGCTCGTCA TGATATCAAT GTTGGCACAA CACAGGCACA
       TCCCTTGGCC CTGACCTTGT TACTGTCACC TCTCGGGTCC TGAGCATTGG TACCTAGTAG TACGAGCAGT ACTATAGTTA CAACCGTGTT GTGTCCGTGT
                                                                                                        PstI
33301  CGTGCATACA CTTCCTCAGG ATTACAAGCT CCTCCCGCGT TAGAACCATA TCCCAGGGAA CAACCCATTC CTGAATCAGC GTAAATCCCA CACTGCAGGG
       GCACGTATGT GAAGGAGTCC TAATGTTCGA GGAGGGCGCA ATCTTGGTAT AGGGTCCCTT GTTGGGTAAG GACTTAGTCG CATTTAGGGT GTGACGTCCC
33401  AAGACCTCGC ACGTAACTCA CGTTGTGCAT TGTCAAAGTG TTACATTCGG GCAGCAGCGG ATGATCCTCC AGTATGGTAG CGCGGGTTTC TGTCTCAAA
       TTCTGGAGCG TGCATTGAGT GCAACACGTA ACAGTTTCAC AATGTAAGCC CGTCGTCGCC TACTAGGAGG TCATACCATC GCGCCCAAAG ACAGAGTTT
33501  GGAGGTAGAC GATCCCTACT GTACGGAGTG CGCCGAGACA ACCGAGATCG TGTTGGTCGT AGTGTCATGC CAAATGGAAC GCCGGACGTA GTCATATTT
       CCTCCATCTG CTAGGGATGA CATGCCTCAC GCGGCTCTGT TGGCTCTAGC ACAACCAGCA TCACAGTACG GTTTACCTTG CGGCCTGCAT CAGTATAAA
```

Figure 15U

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                                          BglII
33601  CTGAAGCAAA ACCAGGTGCG GGCGTGACAA ACAGATCTGC GTCTCCGGTC TCGCCGCTTA GATCGCTCTG TGTAGTAGTT GTAGTATATC CACTCTCTCA
       GACTTCGTTT TGGTCCACGC CCGCACTGTT TGTCTAGACG CAGAGGCCAG AGCGGCGAAT CTAGCGAGAC ACATCATCAA CATCATATAG GTGAGAGAGT
33701  AAGCATCCAG GCGCCCCCTG GCTTCGGGTT CTATGTAAAC TCCTTCATGC GCCGCTGCCC TGATAACATC CACCACCGCA GAATAAGCCA CACCCAGCCA
       TTCGTAGGTC CGCGGGGGAC CGAAGCCCAA GATACATTTG AGGAAGTACG CGGCGACGGG ACTATTGTAG GTGGTGGCGT CTTATTCGGT GTGGGTCGGT
33801  ACCTACACAT TCGTTCTGCG AGTCACACAC GGGAGGAGCG GGAAGAGCTG GAAGAACCAT GTTTTTTTTT TTATTCCAAA AGATTATCCA AAACCTCAAA
       TGGATGTGTA AGCAAGACGC TCAGTGTGTG CCCTCCTCGC CCTTCTCGAC CTTCTTGGTA CAAAAAAAAA AATAAGGTTT TCTAATAGGT TTTGGAGTTT
       BglII
33901  ATGAAGATCT ATTAAGTGAA CGCGCTCCCC TCCGGTGGCG TGGTCAAACT CTACAGCCAA AGAACAGATA ATGGCATTTG TAAGATGTTG CACAATGGCT
       TACTTCTAGA TAATTCACTT GCGCGAGGGG AGGCCACCGC ACCAGTTTGA GATGTCGGTT TCTTGTCTAT TACCGTAAAC ATTCTACAAC GTGTTACCGA
34001  TCCAAAAGGC AAACGGCCCT CACGTCCAAG TGGACGTAAA GGCTAAACCC TTCAGGGTGA ATCTCCTCTA TAAACATTCC AGCACCTTCA ACCATGCCCA
       AGGTTTTCCG TTTGCCGGGA GTGCAGGTTC ACCTGCATTT CCGATTTGGG AAGTCCCACT TAGAGGAGAT ATTTGTAAGG TCGTGGAAGT TGGTACGGGT
34101  AATAATTCTC ATCTCGCCAC CTTCTCAATA TATCTCTAAG CAAATCCCGA ATATTAAGTC CGGCCATTGT AAAAATCTGC TCCAGAGCGC CCTCCACCTT
       TTATTAAGAG TAGAGCGGTG GAAGAGTTAT ATAGAGATTC GTTTAGGGCT TATAATTCAG GCCGGTAACA TTTTTAGACG AGGTCTCGCG GGAGGTGGAA
34201  CAGCCTCAAG CAGCGAATCA TGATTGCAAA AATTCAGGTT CCTCACAGAC CTGTATAAGA TTCAAAAGCG GAACATTAAC AAAAATACCG CGATCCCGTA
       GTCGGAGTTC GTCGCTTAGT ACTAACGTTT TTAAGTCCAA GGAGTGTCTG GACATATTCT AAGTTTTCGC CTTGTAATTG TTTTTATGGC GCTAGGGCAT
34301  GGTCCCTTCG CAGGGCCAGC TGAACATAAT CGTGCAGGTC TGCACGGACC AGCGCGGCCA CTTCCCCGCC AGGAACCATG ACAAAAGAAC CCACACTGAT
       CCAGGGAAGC GTCCCGGTCG ACTTGTATTA GCACGTCCAG ACGTGCCTGG TCGCGCCGGT GAAGGGGCGG TCCTTGGTAC TGTTTTCTTG GGTGTGACTA
                                                         HindIII
34401  TATGACACGC ATACTCGGAG CTATGCTAAC CAGCGTAGCC CCGATGTAAG CTTGTTGCAT GGGCGGCGAT ATAAAATGCA AGGTGCTGCT CAAAAAATCA
       ATACTGTGCG TATGAGCCTC GATACGATTG GTCGCATCGG GGCTACATTC GAACAACGTA CCCGCCGCTA TATTTTACGT TCCACGACGA GTTTTTTAGT
34501  GGCAAAGCCT CGCGCAAAAA AGAAAGCACA TCGTAGTCAT GCTCATGCAG ATAAAGGCAG GTAAGCTCCG GAACCACCAC AGAAAAAGAC ACCATTTTTC
       CCGTTTCGGA GCGCGTTTTT TCTTTCGTGT AGCATCAGTA CGAGTACGTC TATTTCCGTC CATTCGAGGC CTTGGTGGTG TCTTTTTCTG TGGTAAAAAG
34601  TCTCAAACAT GTCTGCGGGT TTCTGCATAA ACACAAAATA AAATAACAAA AAAACATTTA AACATTAGAA GCCTGTCTTA CAACAGGAAA AACAACCCTT
       AGAGTTTGTA CAGACGCCCA AAGACGTATT TGTGTTTTAT TTTATTGTTT TTTTGTAAAT TTGTAATCTT CGGACAGAAT GTTGTCCTTT TTGTTGGGAA
34701  ATAAGCATAA GACGGACTAC GGCCATGCCG GCGTGACCGT AAAAAAACTG GTCACCGTGA TTAAAAAGCA CCACCGACAG CTCCTCGGTC ATGTCCGGAG
       TATTCGTATT CTGCCTGATG CCGGTACGGC CGCACTGGCA TTTTTTTGAC CAGTGGCACT AATTTTTCGT GGTGGCTGTC GAGGAGCCAG TACAGGCCTC
34801  TCATAATGTA AGACTCGGTA AACACATCAG GTTGATTCAC ATCGGTCAGT GCTAAAAAGC GACCGAAATA GCCCGGGGGA ATACATACCC GCAGGCGTAG
       AGTATTACAT TCTGAGCCAT TTGTGTAGTC CAACTAAGTG TAGCCAGTCA CGATTTTTCG CTGGCTTTAT CGGGCCCCCT TATGTATGGG CGTCCGCATC
34901  AGACAACATT ACAGCCCCCA TAGGAGGTAT AACAAAATTA ATAGGAGAGA AAAACACATA AACACCTGAA AAACCCTCCT GCCTAGGCAA AATAGCACCC
       TCTGTTGTAA TGTCGGGGGT ATCCTCCATA TTGTTTTAAT TATCCTCTCT TTTTGTGTAT TTGTGGACTT TTTGGGAGGA CGGATCCGTT TTATCGTGGG
35001  TCCCGCTCCA GAACAACATA CAGCGCTTCC ACAGCGGCAG CCATAACAGT CAGCCTTACC AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC
       AGGGCGAGGT CTTGTTGTAT GTCGCGAAGG TGTCGCCGTC GGTATTGTCA GTCGGAATGG TCATTTTTTC TTTTGGATAA TTTTTTTGTG GTGAGCTGTG
35101  GGCACCAGCT CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA GTATATATAG GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA
       CCGTGGTCGA GTTAGTCAGT GTCACATTTT TTCCCGGTTC ACGTCTCGCT CATATATATC CTGATTTTTT ACTGCATTGC CAATTTCAGG TGTTTTTTGT
35201  CCCAGAAAAC CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC CACAACTTCC TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTCACTTCC
       GGGTCTTTTG GCGTGCGCTT GGATGCGGGT CTTTGCTTTC GGTTTTTTGG GTGTTGAAGG AGTTTAGCAG TGAAGGCAAA AGGGTGCAAT GCAGTGAAGG
```

Figure 15V

pMRKAd5gag MER682

35301 CATTTTAAGA AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA AACCTACGTC ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC
GTAAAATTCT TTTGATGTTA AGGGTTGTGT ATGTTCAATG AGGCGGGATT TTGGATGCAG TGGGCGGGGC AAGGGTGCGG GGCGCGGTGC AGTGTTTGAG

PacI

EcoRI

35401 CACCCCCTCA TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGTTAA TTAAGAATTC GGATCTGCGA CGCGAGGCTG GATGGCCTTC
GTGGGGGAGT AATAGTATAA CCGAAGTTAG GTTTTATTCC ATATAATAAC TACTACAATT AATTCTTAAG CCTAGACGCT GCGCTCCGAC CTACCGGAAG

35501 CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG
GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCGCAACGT CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC

35601 GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA
CGGTCGTTTT CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT

35701 GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG

35801 CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG
GACAGGCGGA AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC

35901 TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC
ACGTGCTTGG GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTC

36001 CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT
GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC CACCGGATTG ATGCCGATGT GATCTTCCTG TCATAAACCA

36101 ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA
TAGACGCGAG ACGACTTCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT

36201 AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT
TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA

36301 TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA
AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG GAAAATTTAG TTAGATTTCA TATATACTCA TTTGAACCAG ACTGTCAATG GTTACGAATT

36401 TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC
AGTCACTCCG TGGATAGAGT CGCTAGACAG ATAAAGCAAG TAGGTATCAA CGGACTGAGG GGCAGCACAT CTATTGATGC TATGCCCTCC CGAATGGTAG

36501 TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT
ACCGGGGTCA CGACGTTACT ATGGCGCTCT GGGTGCGAGT GGCCGAGGTC TAAATAGTCG TTATTTGGTC GGTCGGCCTT CCCGGCTCGC GTCTTCACCA

36601 CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG
GGACGTTGAA ATAGGCGGAG GTAGGTCAGA TAATTAACAA CGGCCCTTCG ATCTCATTCA TCAAGCGGTC AATTATCAAA CGCGTTGCAA CAACGGTAAC

36701 CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA
GATGTCCGTA GCACCACAGT GCGAGCAGCA AACCATACCG AAGTAAGTCG AGGCCAAGGG TTGCTAGTTC CGCTCAATGT ACTAGGGGGT ACAACACGTT

PvuI

36801 AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT
TTTTCGCCAA TCGAGGAAGC CAGGAGGCTA GCAACAGTCT TCATTCAACC GGCGTCACAA TAGTGAGTAC CAATACCGTC GTGACGTATT AAGAGAATGA

36901 GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT
CAGTACGGTA GGCATTCTAC GAAAAGACAC TGACCACTCA TGAGTTGGTT CAGTAAGACT CTTATCACAT ACGCCGCTGG CTCAACGAGA ACGGGCCGCA

Figure 15W

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```
37001  CAACACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT
       GTTGTGCCCT ATTATGGCGC GGTGTATCGT CTTGAAATTT TCACGAGTAG TAACCTTTTG CAAGAAGCCC CGCTTTTGAG AGTTCCTAGA ATGGCGACAA
37101  GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT
       CTCTAGGTCA AGCTACATTG GGTGAGCACG TGGGTTGACT AGAAGTCGTA GAAAATGAAA GTGGTCGCAA AGACCCACTC GTTTTTGTCC TTCCGTTTTA
37201  GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA
       CGGCGTTTTT TCCCTTATTC CCGCTGTGCC TTTACAACTT ATGAGTATGA GAAGGAAAAA GTTATAATAA CTTCGTAAAT AGTCCCAATA ACAGAGTACT
37301  GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT
       CGCCTATGTA TAAACTTACA TAAATCTTTT TATTTGTTTA TCCCCAAGGC GCGTGTAAAG GGGCTTTTCA CGGTGGACTG CAGATTCTTT GGTAATAATA
                                                                                      EcoRI
                                                                              BamHI
37401  CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC AAGAATTGGA TCCGAATTCT TAAT  (SEQ ID NO: 27)
       GTACTGTAAT TGGATATTTT TATCCGCATA GTGCTCCGGG AAAGCAGAAG TTCTTAACCT AGGCTTAAGA ATTA  (SEQ ID NO: 28)
```

Figure 15X

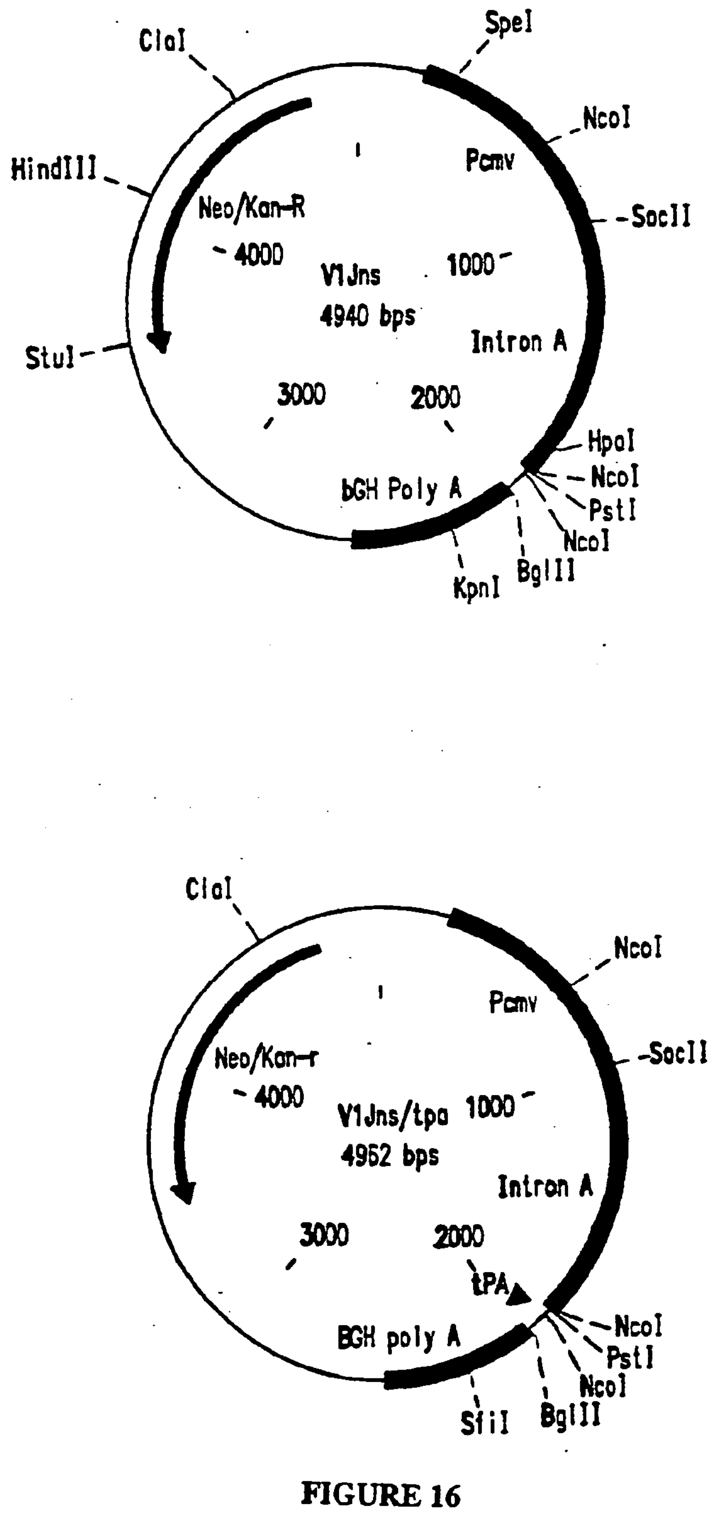

ClaI
SpeI
NcoI
Pcmv
HindIII
-SacII
Neo/Kan-R
4000
V1Jns
4940 bps
1000
StuI
Intron A
3000
2000
HpaI
NcoI
bGH Poly A
PstI
NcoI
BglII
KpnI
ClaI
NcoI
Pcmv
-SacII
Neo/Kan-r
4000
V1Jns/tpa
4962 bps
1000
Intron A
3000
2000
tPA
NcoI
BGH poly A
PstI
NcoI
SfiI
BglII

FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA
BglII MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLy
1 10 20

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL
30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal
60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA
AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy
80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG
sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA
110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC
laPheThrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly
140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA
SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGl
160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC
euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis
220 230

CCCGACAAGTGGACTGTGCAGCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGl
240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC
yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL
270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT
euThrGluValIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis
300 310

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA
GlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCACACCAATGATGTGAAGCAGCTGA
rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT
350 360 370

CTGAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG
hrGluAlaValGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCCCT
GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe
400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
uValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG
430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAAGGTGGTGACCCTGACTGACACCACCAACCAG
luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln
460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl
480 490 500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG
aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG
510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCCACAAGGGCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
luLysValTyrLeuAlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly
540 550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
560 570 580

FIGURE 17B

```
GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGGGGAGG
tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA
               590                            600                            610

CCATGCATGGGCAGGTGGACTGCTCCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG
laMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal
                    620                            630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT
AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
   640                            650                            660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG
uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA
               670                            680                            690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC
laCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn
                    700                            710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT
LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIl
   720                            730                            740

CCACAACTTCAAGAGGAAGGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG
               750                            760                            770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
lnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
                    780                            790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGGTGCCCAG
LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr
   800                            810                            820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCCTCCAGGCAGGATGAGGACT
gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx
               830                            840                            850

AAAGCCCGGGCAGATCT      (SEQ ID NO: 3)
Xx          BglII      (SEQ ID NO: 4)
```

FIGURE 17C

```
GATCACCATGGATGCAATGAAGAGAGGGCTCTCCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGC
        MetAspAlaMetLysArgGlyLeuCysCysValLeuLeuLeuCysGlyAlaValPheValSerP
        -25              -20                                -10

CCAGCGAGATCTCCGCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGC  (within SEQ ID NO: 7)
RoSerGluIleSerAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGly  (within SEQ ID NO: 8)
            -1  2                     10                          20
```

FIGURE 18

```
WT   - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT   -42
OPT  - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC
        M   G   G   K   W   S   K   R   S   V   P   G   W   S   -14

WT   - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT   -84
OPT  - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC
        T   V   R   E   R   M   R   R   A   E   P   A   A   D   -28

WT   - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA   -126
OPT  - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC
        R   V   R   R   T   E   P   A   A   V   G   V   G   A   -42

WT   - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC   -168
OPT  - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC
        V   S   R   D   L   E   K   H   G   A   I   T   S   S   -56

WT   - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA   -210
OPT  - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC
        N   T   A   A   T   N   A   D   C   A   W   L   E   A   -70

WT   - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA   -252
OPT  - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG
        Q   E   D   E   E   V   G   F   P   V   R   P   Q   V   -84

WT   - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC   -294
OPT  - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC
        P   L   R   P   M   T   Y   K   G   A   V   D   L   S   -98

WT   - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC   -336
OPT  - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC
        H   F   L   K   E   K   G   G   L   E   G   L   I   H   -112

WT   - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC   -378
OPT  - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC
        S   Q   K   R   Q   D   I   L   D   L   W   V   Y   H   -126

WT   - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG   -420
OPT  - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC
        T   Q   G   Y   F   P   D   W   Q   N   Y   T   P   G   -140
```

FIGURE 19A

```
WT   - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG   -462
OPT  - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG
        P   G   I   R   F   P   L   T   F   G   W   C   F   K    -154

WT   - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA   -504
OPT  - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG
        L   V   P   V   E   P   E   K   V   E   E   A   N   E    -168

WT   - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG   -546
OPT  - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC
        G   E   N   N   C   L   L   H   P   M   S   Q   H   G    -182

WT   - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC   -588
OPT  - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC
        I   E   D   P   E   K   E   V   L   E   W   R   F   D    -196

WT   - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG   -630
OPT  - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC
        S   K   L   A   F   H   H   V   A   R   E   L   H   P    -210

WT   - GAG TAC TAC AAG GAC TGC TGA  (SEQ ID NO:30)                -651
OPT  - GAG TAC TAC AAG GAC TGC TAA  (contained within SEQ ID NO: 9)
        E   Y   Y   K   D   C        (SEQ ID NO: 10)             -216
```

FIGURE 19B

V1Jns/nef

PstI BglII
CATGGGTCTTTTCTGCAGTCACCGTCCTTGAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC . . . .
M G G K W S K R S V P

SrfI BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC* (SEQ ID NO: 38)
H P E Y Y K D C * (contained within SEQ ID NO: 10)

V1Jns/nef(G2A.LLAA)

PstI BglII
CATGGGTCTTTTCTGCAGTCACCGTCCTTGAGATCTGCCACC ATG GCC GGC AAG TGG TCC AAG AGG TCC GTG CCC . . . .
M A G K W S K R S V P

SrfI BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC* (SEQ ID NO: 39)
H P E Y Y K D C * (contained within SEQ ID NO:14)

V1Jns/tpanef & V1Jns/tpanef(LLAA)

PstI
CATGGGTCTTTTCTGCAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG
M D A M K R G L C C V

BglII
CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ATC TCC TCC AAG AGG TCC GTG CCC . . . .
L L L C G A V F V S P S E I S S K R S V P

SrfI BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA *AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC* (SEQ ID NO: 40)
H P E Y Y K D C * (contained withon SEQ ID NO: 16)

FIGURE 20

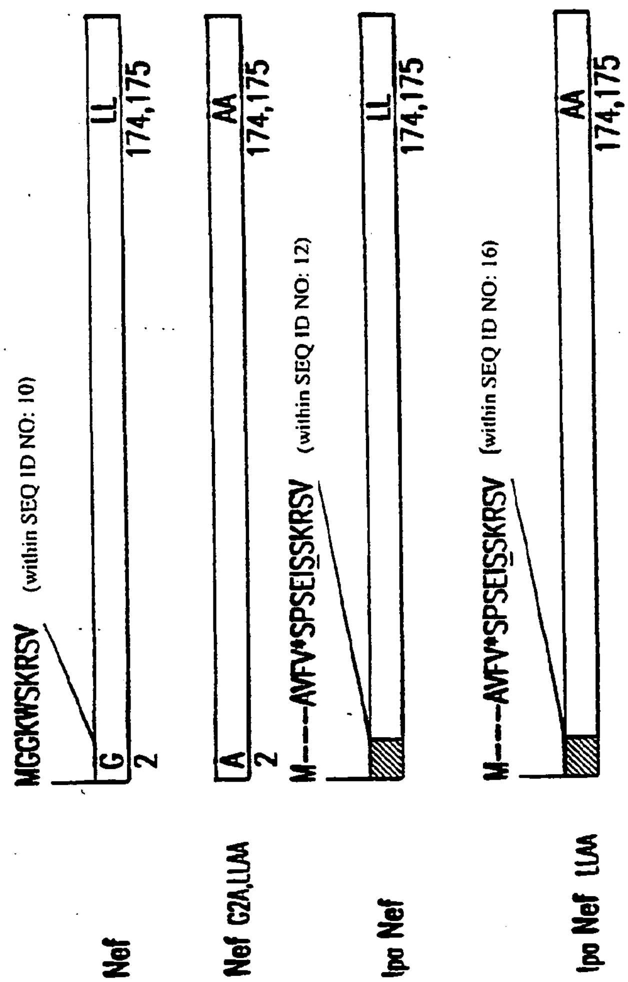

Nef
MGGKWSKRSV (within SEQ ID NO: 10)
G
2
LL
174,175
Nef G2A,LLAA
A
2
AA
174,175
Ipa Nef
M----AVFV*SPSEISSKRSV (within SEQ ID NO: 12)
LL
174,175
Ipa Nef LLAA
M----AVFV*SPSEISSKRSV (within SEQ ID NO: 16)
AA
174,175

FIGURE 21

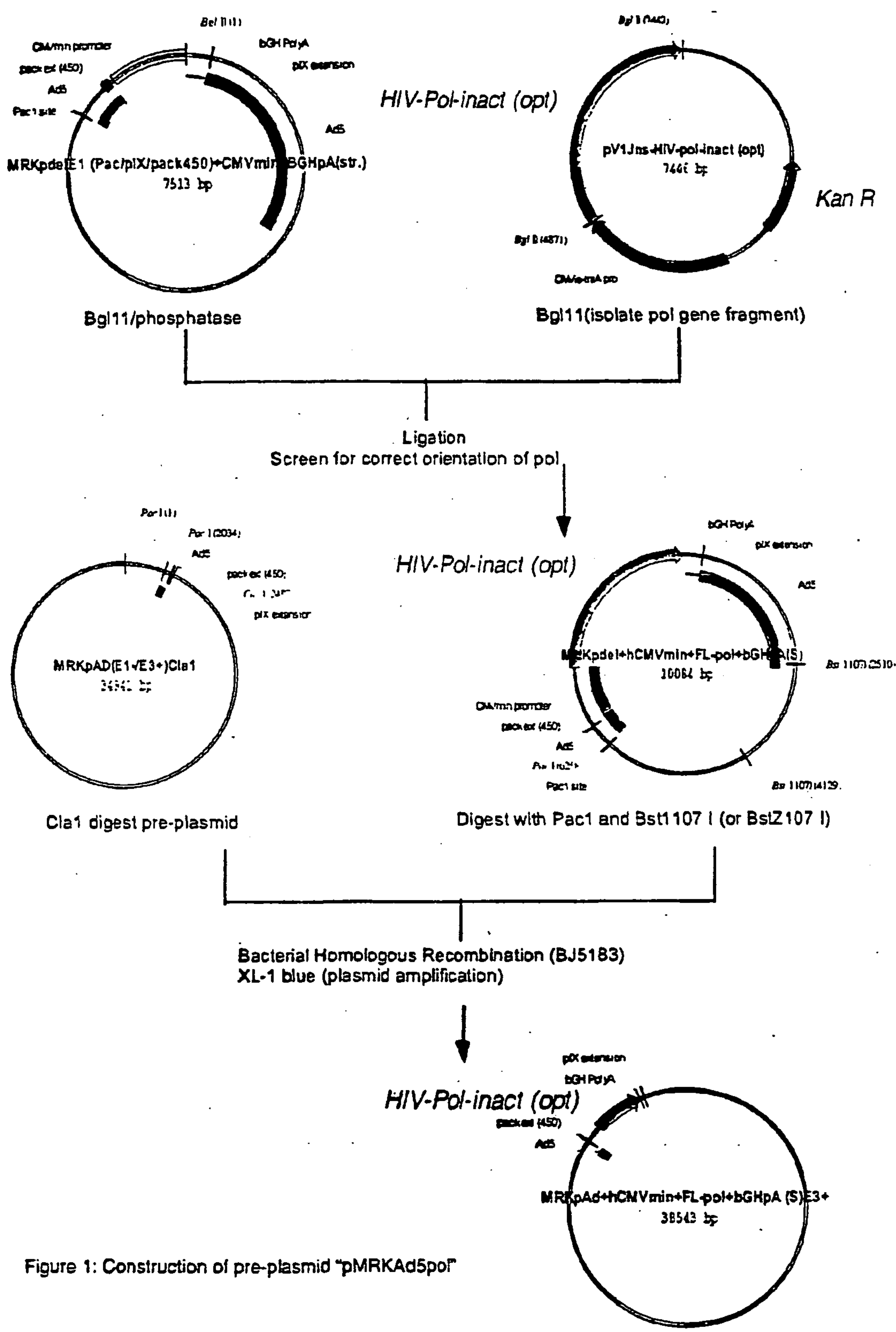


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

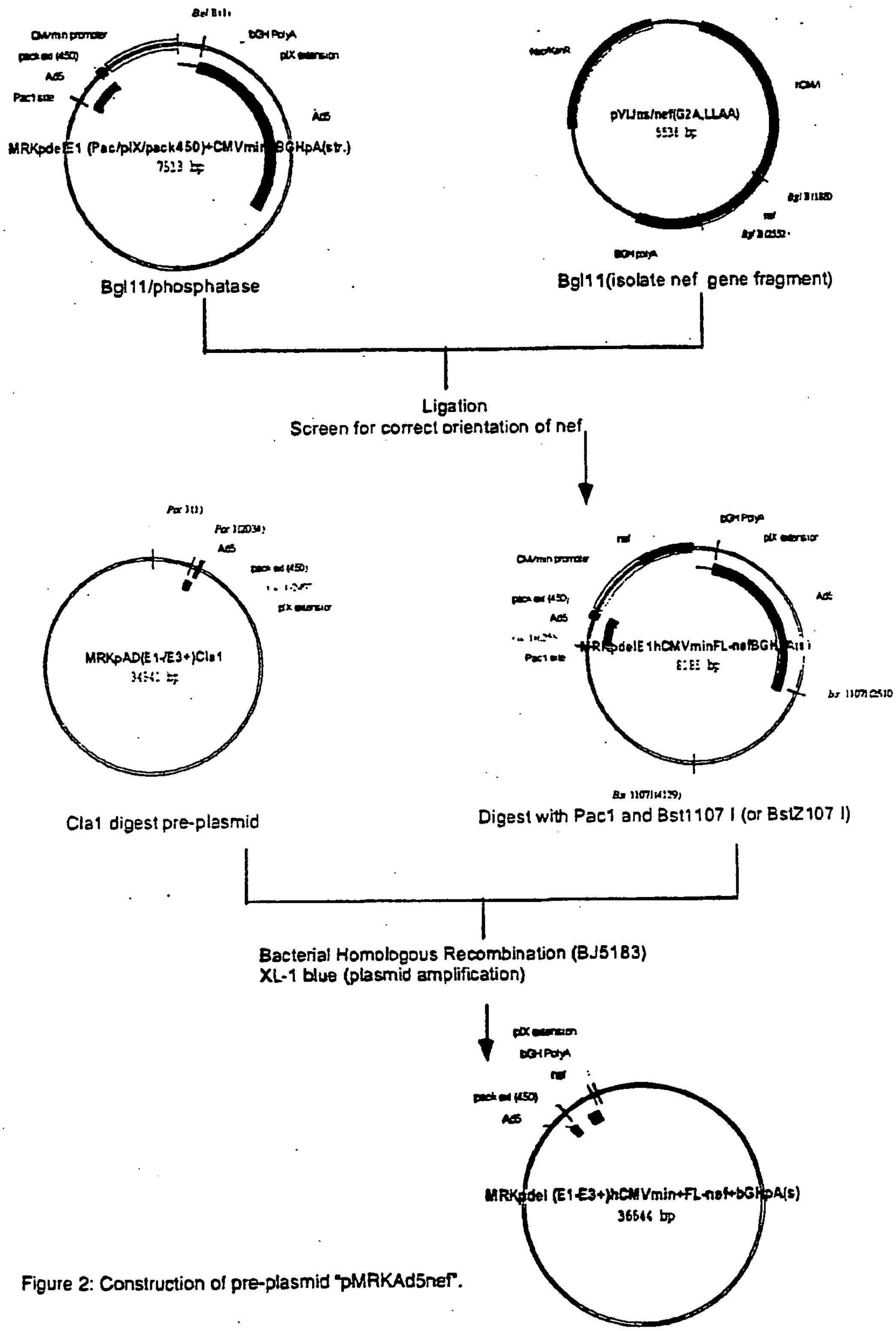
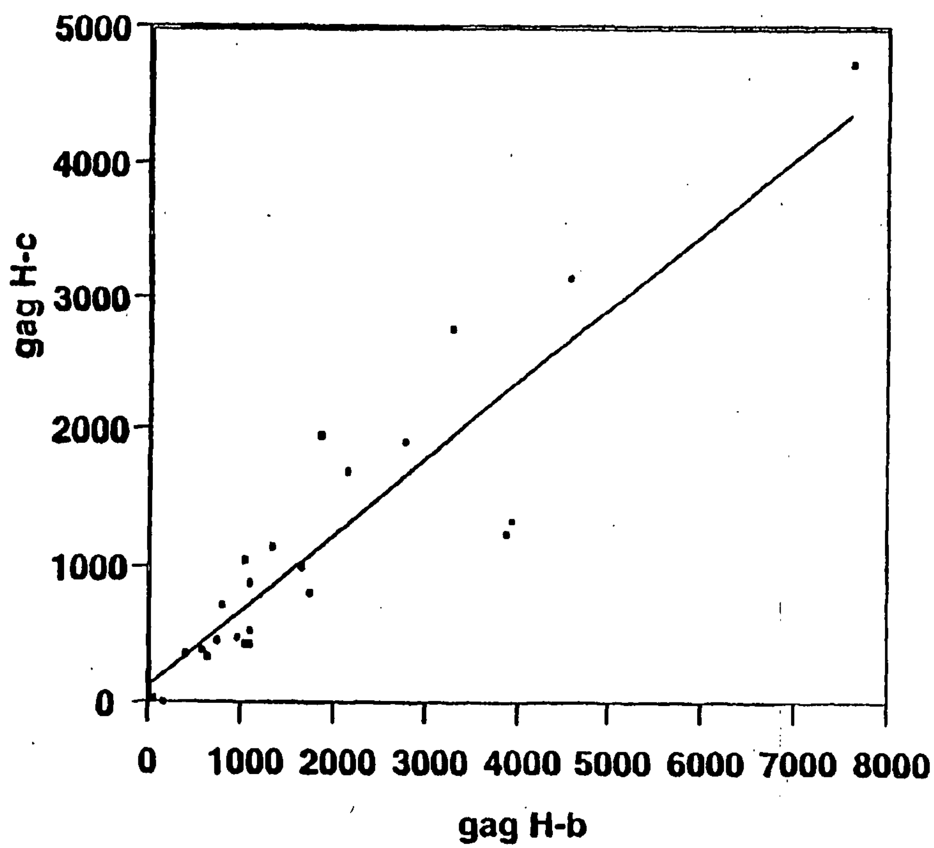


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

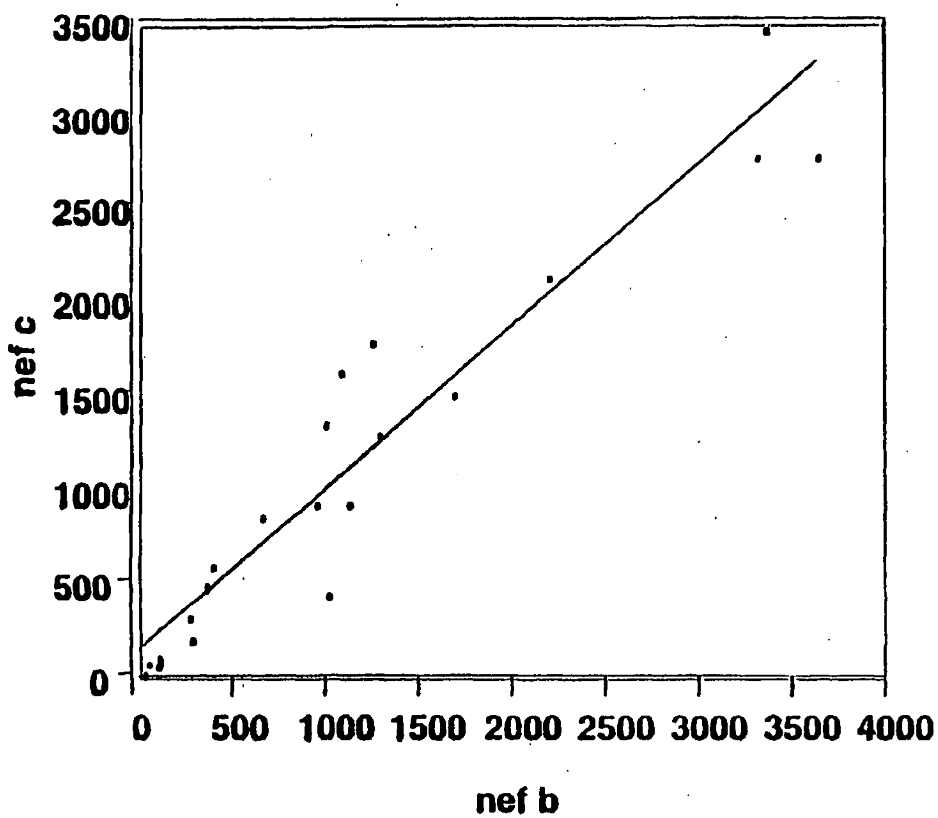


Linear Fit

gag H-c = 111.603 + 0.55866 gag H-b

Summary of Fit	
RSquare	0.816775
RSquare Adj	0.80914
Root Mean Square Error	474.9639
Mean of Response	1158.115
Observations (or Sum Wgts)	26

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



nef c = 131.132 + 0.8646 nef b

Summary of Fit	
RSquare	0.91685
RSquare Adj	0.91289
Root Mean Square Error	289.7718
Mean of Response	1096.435
Observations (or Sum Wgts)	23

FIGURE 25

MRKAd5pol MER1062
(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

```
  1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
    GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

 51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
    CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
    ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
    CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
    CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA
    GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
    TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
    CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
    GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
    CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
    ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
    ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
    ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
    GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
    TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
    TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
    GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
    GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT
```

Figure 26A

901 TCGCTATTAC C[redacted]GGTGATG CGGTTTTGGC AGTACATCAA TGGGCG[redacted]GA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
GGTAGTTCTT CTTCCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG ACACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26B

```
1851  TGGGCAGCAC A[redacted]CCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTG[redacted]T
      ACCCGTCGTG TCCTGGTTCT AACTCCTCGA CTCCGTCGTG GACGACTCCA

1901  GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG
      CCCCGGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC

1951  TGGATGGGCT ATGAGCTGCA CCCCGACAAG TGGACTGTGC AGCCCATTGT
      ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA

2001  GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG
      CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC

2051  GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
      CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC

2101  CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT
      GACACGTTCG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA

2151  GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG
      CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC

2201  AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG
      TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC

2251  ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC
      TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTTAGA TGGTCCTCGG

2301  CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA
      GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCCGGGTGT

2351  CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
      GGTTACTACA CTTCGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC

2401  TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA
      AGGTAACACT AGACCCCGTT CTGGGGGTTC AAGTTCGACG GGTAGGTCTT

2451  GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC
      CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG

2501  CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG
      GACTCACCCT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC

2551  CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC
      GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGAAGATAC ACCGACCCCG

2601  TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG
      ACGGTTGTCC CTCTGGTTCG ACCCGTTCCG ACCGATACAC TGGTTGTCCC

2651  GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
      CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG

2701  CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT
      GAGGTCCGGT AGATGGACCG GGAGGTCCTG AGACCGGACC TCCACTTGTA

2751  TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC
      ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG
```

Figure 26C

```
2801  AGTCTGAGTC T[illegible]CTGGTG AACCAGATCA TTGAGCAGCT GATCAA[illegible]G
      TCAGACTCAG ACTCGACCAC TTGGTCTAGT AACTCGTCGA CTAGTTCTTC

2851  GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA
      CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901  TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC
      ACTCGTCCAC CTGTTCGACC ACAGACGACC GTAGTCCTTC CACGACAAGG

2951  TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
      ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG

3001  TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA
      ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCCT

3051  GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG
      CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101  GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG
      CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151  GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA
      CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAACT

3201  GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC
      CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251  TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
      ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301  TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT
      AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351  CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG
      GTTCGTCCTC AAACCGTAGG GGATGTTGGG GGTCAGGGTC CCCCACCACC

3401  CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG
      GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC

3451  GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACTT
      CGACTCGTGG ACTTCTGTCG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501  CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG
      GTTCTCCTTC CCCCCGTAGC CCCCGATGAG GCGACCCCTC TCCTAACACC

3551  ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
      TGTAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG

3601  AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG
      TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCCT TGGGGGACAC

3651  GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC
      CTTCCCGGGA CGGTTCGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG

3701  AGGACAACTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC
      TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG
```

Figure 26 D

3751 AGGGACTATG G[illegible]AGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCA[illegible]CA
TCCCTGATAC C[illegible]TCGTCTA CCGACCCCTA CTGACACACC GGAGGT[illegible]GT

3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
CCTACTCCTG ATTTCGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG

3851 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACTGGGA CCTTCCACGG

3901 ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT
TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC

4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051 ATGGCCGATC GGCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCCAC

4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC
CCTTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA
TCGTCGGCGG CGGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAAACTCTAC
CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT
ATGGAACTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351 CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
GGCGGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC
AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501 GGGAACTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT
CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCGTCCAA

4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA
AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601 AAAACCAGAC TCTGTTTGGA TTTGGATCAA GCAAGTGTCT TGCTGTCTTT
TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTCG
TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCCAGC

Figure 26E

```
4701  TTGAGGGTCC TGTGTATTTT TTCCAGGACG TGGTAAAGGT GACTCTGGAT
      AACTCCCAGG A[illegible]ATAAAA AAGGTCCTGC ACCATTTCCA CTGAGA[illegible]A

4751  GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCACT
      CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA

4801  GCAGAGCTTC ATGCTGCGGG GTGGTGTTGT AGATGATCCA GTCGTAGCAG
      CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851  GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC
      CTCGCGACCC GCACCACGGA TTTTTACAGA AAGTCATCGT TCGACTAACG

4901  CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTTA AGCTGGGATG
      GTCCCCGTCC GGGAACCACA TTCACAAATG TTTCGCCAAT TCGACCCTAC

4951  GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
      CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001  GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC
      CGATACAAGG GTCGGTATAG GGAGGCCCCT AAGTACAACA CGTCTTGGTG

5051  CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG
      GTCGTGTCAC ATAGGCCACG TGAACCCTTT AAACAGTACA TCGAATCTTC

5101  GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
      CTTTACGCAC CTTCTTGAAC CTCTGCGGGA ACACTGGAGG TTCTAAAAGG

5151  ATGCATTCGT CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
      TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCGCC GCCGGACCCG

5201  GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT
      CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251  CGTCATAGGC CATTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT
      GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCCACGG TCTGACGCCA

5301  ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT
      TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351  TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TGCGGGGCGA
      AAGGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT

5401  TGAAGAAAAC GGTTTCCGGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
      ACTTCTTTTG CCAAAGGCCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC

5451  TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCGT AAATCACACC
      AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG

5501  TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
      ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551  TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
      ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601  CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG
      GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC
```

Figure 26F

5651 CAAGGAAGCA A[redacted]TTTTTCA ACGGTTTGAG ACCGTCCGCC GTAGGC[redacted]C
GTTCCTTCGT TTCAAAAAGT TGCCAAACTC TGGCAGGCGG CATCCGTACG

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC
AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG

5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC

5801 CGGCTTTCGC TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA

5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG
GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC

5901 TGAAGGGGTG CGCTCCGGGC TGCGCGCTGG CCAGGGTGCG CTTGAGGCTG
ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC

5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG
CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC

6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA

6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA
ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT

6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCTCAT

6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG
CCGTAGGCGC GGCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC

6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCCC ATGCTTTTTG
ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC

6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC
TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG

6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTCGA
CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT

6351 GCGGTGTTCC GCGGTCCTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA
CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT

6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCACCC TCCCCATCGC

6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT
CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACACT TCTGTGTACA

6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC

6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC
ACTGGCCCAC AAGGACTTCC CCCCGATATT TTCCCCCACC CCCGCGCAAG

Figure 26G

6601 GTCCTCACTC TCTTCCGCAT CGCTGTCTGC GAGGGCCAGC TGTTGCGGTG
CAGGAGTGAG AGAAGGCGTA GCGACAGACG CTCCCGGTCG ACAACGCCAC

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT
TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTC TAACAGTCAA

6701 TCCAAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT
AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA

6751 GAGGGTGGCC GCATCCATCT GGTCAGAAAA GACAATCTTT TTGTTGTCAA
CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT

6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG
CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC

6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT
CTCGCGTCCC AAACCAAAAA CAGCGCTAGC CGCGCGAGGA ACCGGCGCTA

6901 GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTCG GGAAAGACGG
CAAATCGACG TGCATAAGCG CGCGTTGCGT GGCGGTAAGC CCTTTCTGCC

6951 TGGTGCGCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG
ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC

7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT
CACTGTTCCA GTTGCGACCA CCGATGGAGA GGCGCATCCG CGAGCAACCA

7051 CCAGCAGAGG CGGCCGCCCT TGCGCGAGCA GAATGGCGGT AGGGGGTCTA
GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCCAGAT

7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC
CGACGCAGAG CAGGCCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTCG

7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG
TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC
GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCCTG

7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTCG
GGGTACCGTA CCCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC

7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT
ATTTGCATCT CCCCGAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA

7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCG TGCGAGGGAG
AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGCTCCCTC

7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG
GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC

7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG
TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG
CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7551 AGGCGTAGGA GTCGCGCAGC TTGTTGACCA GCTCGGCGGT GACCTGCACG
TCCGCATCCT CAGCGCGTCG AACAACTGGT CGAGCCGCCA CTGGACGTGC

7601 TCTAGGGCGC AGTAGTCCAG GGTTTCCTTG ATGATGTCAT ACTTATCCTG
AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC

7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAAACTCT TCGCGGTCTT
AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA

7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA

7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG

7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG
CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC

7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
GTTTCCACAG GGACTGGTAC TGAAACTCCA TGACCATAAA CTTCAGTCAC

7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTTGGA
AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT

7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC

8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT

8051 CGGTTGTTAA TTACCTGGGC GGCGAGCACG ATCTCGTCAA AGCCGTTGAT
GCCAACAATT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA

8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAACTACC

8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
TTCCGTTAAA AAATTCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG

8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT

8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTCC

8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC

8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCG CGGCTAGGTC
CATTCGCCCA GAACAAGGGT CGCCAGGGTA GGTTCCAAGC GCCGATCCAG

8401 TCGCGCGGCA GTCACTAGAG GCTCATCTCC GCCGAACTTC ATGACCAGCA
AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT

8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
ACTTCCCGTG CTCGACGAAG GGTTTCCGGG GGTAGGTTCA TATCCAGAGA

Figure 26I

8501 ACATCGTAGG T[illegible]AAAGAG ACGCTCGGTG CGAGGATGCG AGCCGA[illegible]G
TGTAGCATCC ACTGTTTCTC TGCGAGCCAC GCTCCTACGC TCGGCTAGCC

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTAA
CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG
TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT
CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCTTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCGGCTGC TTGTCCTTGA
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA
GCTCGGGTTT CAGGTCTACA GGCGCGCGCC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

9101 GGCGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG
CCGCCCGCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG
ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GGCGGCCCTC

9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG
TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCCTGAATC
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAACTA GAGGACTTAG

9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCG GTGAGCTTGA ACCTGAAAGA
ACCGCGGAGA CGCACTTCTG CTGCCCGGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGCC TGGCGCAAAA
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT

9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9451 TGCTCGATCT C[illegible]CTCCTG GAGATCTCCG CGTCCGGCTC GCTCCA[illegible]T
ACGAGCTAGA GAAGGAGGAC CTCTAGAGGC GCAGGCCGAG CGAGGTGCCA

9501 GGCGGCGAGG TCGTTGGAAA TGCGGGCCAT GAGCTGCGAG AAGGCGTTGA
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA
GCCCGCGCGT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCG
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
AACTATAGGG GGTTCCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGCGAAGTTG AAAAACTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
CCGCTTCAAC TTTTTGACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC
AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA
TTTGGAGAGC TCTTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG
CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GGCGGATGGT
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

```
10401  CGACAGAAGC A[illegible]TGTCCT TGGGTCCGGC CTGCTGAATG CGCAGG[illegible]CT
       GCTGTCTTCG T[illegible]TACAGGA ACCCAGGCCG GACGACTTAC GCGTCC[illegible]CA

10451  CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG
       GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC

10501  TCTTGCATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTCC
       AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG

10551  TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT
       ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAAA CCGGCATCCA

10601  GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA
       CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT

10651  AGCAGGGCTA GGTCGGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC
       TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG

10701  CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CGGTGGTATG
       GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC

10751  CGCCCGTGTT GATGGTGTAA GTGCAGTTGG CCATAACGGA CCAGTTAACG
       GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC

10801  GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC
       CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG

10851  CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC
       GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG

10901  CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG
       GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCCAC

10951  GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA
       CGGCCCCGAG GCCCCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT

11001  GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG
       CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC

11051  GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC
       CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG

11101  ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA
       TACCAGCCCT GCGAGACCGG CCAGTCCGCG CGCGTTAGCA ACTGCGAGAT

11151  GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG
       CTGGCACGTT TTCCTCTCGG ACATTCGCCC GTGAGAAGGC ACCAGACCAC

11201  GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA
       CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT

11251  TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA
       AGGCCGGCAG GCGGCACTAG GTACGCCAAT GGCGGGCGCA CAGCTTGGGT

11301  GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG
       CCACACGCTG CAGTCTGTTG CCCCCTCACG AGGAAAACCG AAGGAAGGTC
```

Figure 26L

```
11351  GCGCGGCGGC TGCTGCGCTA GCTTTTTTGG CCACTGGCCG CGCGCAGCGT
       CGCGCCGCCG ACGACGCGAT CGAAAAAACC GGTGACCGGC GCGCGTCGCA

11401  AAGCGGTTAG GCTGGAAAGC GAAAGCATTA AGTGGCTCGC TCCCTGTAGC
       TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451  CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT
       GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501  CGGACCGGCC GGACTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG
       GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTC

11551  ACCCCGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTTTGCTT
       TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601  TTCCCAGATG CATCCGGTGC TGCGGCAGAT GCGCCCCCCT CCTCAGCAGC
       AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCG

11651  GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCTC CCCTCCTCCT
       CCGTTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701  ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA
       TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751  TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG
       AATGCTTGGG GGCGCCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801  GCGAGGGCCT GGCGCGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG
       CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTCC

11851  GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT
       CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901  GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT
       CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951  TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG
       AGGTGCGTCC CGCGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001  CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG
       GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051  CGCACACGTG GCGGCCGCCG ACCTGGTAAC CGCATACGAG CAGACGGTGA
       GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101  ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT
       TGGTCCTCTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151  GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT
       CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201  AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT
       TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CGCGTCGACA

12251  TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTCAG GGATGCGCTG
       AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC
```

Figure 26 M

12301 CTAAACATAG T[illegible]GCCCGA GGGCCGCTGG CTGCTCGATT TGATAA[illegible]T
GATTTGTATC ATCTCGGGCT CCCGGCGACC GACGAGCTAA ACTATTTGTA

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG
GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCGC
ACCGGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCCATA GACAAGGAGG TAAAGATCGA
TTCTATATGG TATGGGGAAT GCAAGGGTAT CTGTTCCTCC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC
CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG
ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT
GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTTCCCGGGA

12651 GGCTGGCACG GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG
CCGACCGTGC CCGTCGCCGC TATCTCTCCG GCTCAGGATG AAACTGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG
CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG
CGGCCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT
GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG
TGATTCGCCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC

12901 CGGTGCGGGC GGCGCTGCAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC
GCCACGCCCG CCGCGACGTC TCGGTCGGCA GGCCGGAATT GAGGTGCCTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC
CTGACCGCGG TCCAGTACCT GGCGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG
ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG
TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCG ACGAGGCCGG
TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA
GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTCGCCGT

13201 ACGTGCAGAC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG
TGCACGTCTG GTTGGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

```
13251  GCGCAGCGTG AGCGCGCGCA GCAGCAGGGC AACCTGGGCT CCATGGTTGC
       CGCGTCGCAC TCGCGCGCGT CGTCGTCCCG TTGGACCCGA GGTACCAACG

13301  ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG
       TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGCACGGC GCCCCTGTCC

13351  AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA
       TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401  CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG
       GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451  TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAACTTGC
       ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTTGAACG

13501  AGGGGCTGTG GGGGGTGCGG GCTCCCACAG GCGACCGCGC GACCGTGTCT
       TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA

13551  AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT
       TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601  CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA
       GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651  CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC
       GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701  CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG
       GTCCTCTAAT GTTCACAGTC GGCGCGCGAC CCCGTCCTCC TGTGCCCGTC

13751  CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC
       GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801  CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG
       GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC

13851  CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT
       GTCGTCTCGC ACTCGGAATT GGACTACGCG CTGCCCCATT GCGGGTCGCA

13901  GGCGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA
       CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951  ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCATCG CGCGGCCGCC
       TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001  GTGAACCCCG AGTATTTCAC CAATGCCATC TTGAACCCGC ACTGGCTACC
       CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051  GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCGAG GGTAACGATG
       CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101  GATTCCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG
       CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAAGGGG CGTTGGCGTC

14151  ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA
       TGGGACGATC TCAACGTTGT CGCGCTCGTC CGTCTCCGCC GCGACGCTTT
```

Figure 260

```
14201  GGAAAGCTTC C[illegible]AGGCCAA GCAGCTTGTC CGATCTAGGC GCTGCG[illegible]CC
       CCTTTCGAAG G[illegible]TCCGGTT CGTCGAACAG GCTAGATCCG CGACGC[illegible]GG

14251  CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC
       GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301  AGCACTCGCA CCACCCGCCC GCGCCTGCTG GGCGAGGAGG AGTACCTAAA
       TCGTGAGCGT GGTGGGCGGG CGCGGACGAC CCGCTCCTCC TCATGGATTT

14351  CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTC
       GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401  CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG
       GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451  TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG
       ATGCGCGTCC TCGTGTCCCT GCACGGTCCG GGCGCGGGCG GGTGGGCAGC

14501  TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG
       AGTTTCCGTG CTGGCAGTCG CCCCAGACCA CACCCTCCTG CTACTGAGCC

14551  CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG
       GTCTGCTGTC GTCGCAGGAC CTAAACCCTC CCTCACCGTT GGGCAAACGC

14601  CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAAAAA AAAAGCATGA
       GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651  TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTTCTT
       ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701  GTATTCCCCT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCCTCCT
       CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751  CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG
       GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCACCGCC GCCGCGACCC

14801  TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC
       AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851  TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC
       ACGCCGGATG GCCCCCCTCT TTGTCGTAGG CAATGAGACT CAACCGTGGG

14901  CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT
       GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCTACA

14951  GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA
       CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001  TTCAAAACAA TGACTACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT
       AAGTTTTGTT ACTGATGTCG GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051  CTTGACGACC GGTCGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC
       GAACTGCTGG CCAGCGTGAC CCCGCCGCTG GACTTTTGGT AGGACGTATG

15101  CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC
       GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCCGCG
```

Figure 26 P

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15151  GGGTGATGGT G[redacted]CGCTTG CCTACTAAGG ACAATCAGGT GGAGCT[redacted]A
       CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201  TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT
       ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251  GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG
       CTGGTATCTG GAATACTTGT TGCGCTAGCA CCTCGTGATG AACTTTCACC

15301  GCAGACAGAA CGGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC
       CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351  CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG
       GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401  GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT
       CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAAC GACGGTCCTA

15451  GCGGGGTGGA CTTCACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC
       CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501  AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA
       TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551  GGGTGGTAAC ATTCCCGCAC TGTTGGATGT GGACGCCTAC CAGGCGAGCT
       CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601  TGAAAGATGA CACCGAACAG GGCGGGGGTG GCGCAGGCGG CAGCAACAGC
       ACTTTCTACT GTGGCTTGTC CCGCCCCCAC CGCGTCCGCC GTCGTTGTCG

15651  AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA
       TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701  GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA
       CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751  CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC
       GTGCCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801  GCCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACCGGTGAT
       CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851  CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA
       GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCGT

15901  ATGACAGCAC CTTCACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC
       TACTGTCGTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951  GGCGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA
       CCGCTGGGAG TCTGGCCTTA GGCGAGTACC TGGGACGAAA CGTGAGGACT

16001  CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC
       GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051  AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG
       TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC
```

Figure 26 Q

16101 GTGGGCGCCG A[illegible]TGTTGCC CGTGCACTCC AAGAGCTTCT ACAACG[illegible]CA
CACCCGCGGC T[illegible]ACAACGG GCACGTGAGG TTCTCGAAGA TGTTGC[illegible]GT

16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT
CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC
AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251 ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT
TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG
TGGCGACGCG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC

16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG
GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401 CCGCGCGTCC TATCGAGCCG CACTTTTTGA GCAAGCATGT CCATCCTTAT
GGCGCGCAGG ATAGCTCGGC GTGAAAAACT CGTTCGTACA GGTAGGAATA

16451 ATCGCCCAGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT
TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA

16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG
AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC

16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC
GTGATGGCGC GCGGGACCCC GCGCGTGTTT GCGCCGGCGT GACCCGCGTG

16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA
GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT

16651 CGCCCACGCC GCCACCAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG
GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC

16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT
CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751 AGCACGTCGC CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG
TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCCGCC

16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGGC GGCCATGCGG
GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC

16851 GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG
CGGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GGTCCAGGTC

16901 GCGACGAGCG GCCGCCGCAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG
CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951 GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC
CAGCGTCCCC GTTGCACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG

17001 GTGCCCGTGC GCACCCGCCC CCCGCGCAAC TAGATTGCAA GAAAAAACTA
CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26R

17051 CTTAGACTCG TACTGTTGTA TGTATCCAGC GGCGGCGGCG CGCAACGAAG
GAATCTGAGC ATGACAACAT ACATAGGTCG CCGCCGCCGC GCGTTGCTTC

17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG
GATACAGGTT CGCGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCCCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA
CTCTAGATAC CGGGGGGCTT CTTCCTTCTC GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG
CGATTTCGCC CAGTTTTTCT TTTTCTTTCT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG
TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTCGAC GCGTAAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT
TTTCCAGCTG CGCATTTTGC ACAAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCGGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG
ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG
ACATGCCGCT GCTCCTGGAC GAACTCGTCC GGTTGCTCGC GGAGCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA
AAACGGATGC CTTTCGCCGT ATTCCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC
CCCGTTGGGT TGTGGATCGG ATTTCGGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT
GGCGCGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTCGC GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA
CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC
TCTACAGAAC CTTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG
CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTCAGA TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA
CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GGCGGTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCGGTTGC CTCAGCGGTG GCGGATGCCG
CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CGGTGCAGGC GGTCGCTGCG GCCGCGTCCA AGACCTCTAC GGAGGTGCAA
GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCGGCGCC CGCGCCGTTC
TGCCTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGGCAAG

17951 GAGGAAGTAC GGCGCCGCCA GCGCGCTACT GCCCGAATAT GCCCTACATC
CTCCTTCATG CCGCGGCGGT CGCGCGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18001 CTTCCATTGC GCCTACCCCC GGCTATCGTG GCTACACCTA CCGCCGCAGA
GAAGGTAACG CGGATGGGGG CCGATAGCAC CGATGTGGAT GGCGGCGTCT

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG
TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC
AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCCAGC
CGCTTCCTCC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTCG

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG
TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA
GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGCGCACCAC
CCCCGTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCCT
GCCGCCGCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT
GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG
GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTTGTTC AACGTACACC

18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC
TTTTTAGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC
ATAAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACTGGCAA GATATCGGCA CCAGCAATAT
CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTCGTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT
CTCGCCACCG CGGAAGTCGA CCCCGAGCGA CACCTCGCCG TAATTTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGGA AGGCCTGGAA CAGCAGCACA
AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT
CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC
CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA
TCCGTCACGT TTTATTCTAA TTGTCATTCG AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCGTGGCGA
CTCCTCGGAG GTGGCCGGCA CCTCTGTCAC AGAGGTCTCC CCGCACCGCT

Figure 26T

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18951  AAAGCGTCCG C[illegible]CCGACA GGGAAGAAAC TCTGGTGACG CAAATA[illegible]G
       TTTCGCAGGC GC[illegible]GGGCTGT CCCTTCTTTG AGACCACTGC GTTTATC[illegible]C

19001  AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT
       TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051  CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC
       GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101  GCTGGACCTG CCTCCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG
       CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151  GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC
       CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201  GCCGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA
       CGGCGGTCGC CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251  AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC
       TTCGTGTGAC TTGTCGTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301  GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC
       CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351  ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA
       TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401  TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC
       ACCGATGGGG AAGCTACTAC GGCGTCACCA GAATGTACGT GTAGAGCCCG

19451  CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCAGT TTGCCCGCGC
       GTCCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501  CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG
       GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551  CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG
       GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAAA CTGCGACGCC

19601  TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT
       AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651  CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT
       GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701  TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT
       AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751  GGCACTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA
       CCGTGACGGA TGTTGCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801  ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG
       TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851  ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAACTCAC
       TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTTGAGTG
```

Figure 26 U

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19901  GTATTTGGGC A[illegible]GCCTTA TTCTGGTATA AATATTACAA AGGAGG[illegible]T
       CATAAACCCG TCCGCGGAAT AAGACCATAT TTATAATGTT TCCTCCCATA

19951  TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTC
       AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG

20001  AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT
       TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTTG TCTTTAATTA

20051  CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA
       GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT

20101  CGGTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG
       GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAC

20151  TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC
       ATTTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201  TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT
       AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTCA

20251  GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCCAGAC ACTCATATTT
       CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301  CTTACATGCC CACTATTAAG GAAGGTAACT CACGAGAACT AATGGGCCAA
       GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT

20351  CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT
       GTTAGATACG GGTTGTCCGG ATTAATGTAA CGAAAATCCC TGTTAAAATA

20401  TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC
       ACCAGATTAC ATAATGTTGT CGTGCCCATT ATACCCACAA GACCGCCCGG

20451  AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG
       TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCTGTC TTTGTGTCTC

20501  CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT
       GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551  TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA
       AAGATACACC TTAGTCCGAC AACTGTCGAT ACTAGGTCTA CAATCTTAAT

20601  TTGAAAATCA TGGAACTGAA GATGAACTTC CAAATTACTG CTTTCCACTG
       AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC

20651  GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG
       CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701  TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG
       AGTCCTTTTA CCTACCCTTT TTCTACGATG TCTTAAAAGT CTATTTTTAC

20751  AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC
       TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG

20801  CTGTGGAGAA ATTTCCTGTA CTCCAACATA GCGCTGTATT TGCCCGACAA
       GACACCTCTT TAAAGGACAT GAGGTTGTAT CGCGACATAA ACGGGCTGTT
```

Figure 26 V

20851 GCTAAAGTAC A[illegible]CCTTCCA ACGTAAAAAT TTCTGATAAC CCAAACA[illegible]CT
CGATTTCATG T[illegible]GGAAGGT TGCATTTTTA AAGACTATTG GGTTTG[illegible]GA

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC
TGCTGATGTA CTTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC
TAATTGGAAC CTCGTGCGAC CAGGGAACTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG
TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT
CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA
CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC
GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC
ATTCCCAACT GCCTCGGTCG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT
TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA
ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC
TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTGCACGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA
GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT
CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC
TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA
AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG
ACTGGCGGAC GAATGGGGGT TGCTCAAACT TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCTG
CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC
CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA
TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT

Figure 26 W

```
21801  TGAGCCGTCA G[illegible]TGGTGGAT GATACTAAAT ACAAGGACTA CCAACA[illegible]TG
       ACTCGGCAGT C[illegible]CCACCTA CTATGATTTA TGTTCCTGAT GGTTGT[illegible]AC

21851  GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC
       CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901  CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA
       GTGGTACGCG CTTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951  TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT
       ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAACGCTA

22001  CGCACCCTTT GGCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC
       GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051  ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCCACGCGC
       TGAGTGTCTG GACCCGGTTT TGGAAGAGAT GCGGTTGAGG CGGGTGCGCG

22101  TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCCAC CCTTCTTTAT
       ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151  GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG
       CAAAACAAAC TTCAGAAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201  CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA
       GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT

22251  CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA
       GTTGTATTTC TTCGTTCGTT GTAGTTGTTG TCGACGGCGG TACCCGAGGT

22301  GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGGTTG TGGGCCATAT
       CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351  TTTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA
       AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401  GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC
       CGAGCGGACG CGGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGCATG

22451  ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT
       TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTTGTAC GATGGAGAAA

22501  GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA
       CTCGGGAAAC CGAAAAGACT GGTCGCTGAG TTCGTCCAAA TGGTCAAACT

22551  GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT
       CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601  GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC
       CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651  GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC
       CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTTGACCGG

22701  CCAAACTCCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC
       GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTTGGAATAA TGGCCCCATG
```

Figure 26 X

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22751  CCAACTCCAT GCTCAACAGT CCCCAGGTAC AGCCCACG[illegible] [illegible]AC
       GGTTGAGGTA C[illegible]TTGTCA GGGGTCCATG TCGGGTGGGA CGCAGC[illegible]G

22801  CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCCGCAG
       GTCCTTGTCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851  CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTTGTCAC TTGAAAAACA
       GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAAACAGTG AACTTTTTGT

22901  TGTAAAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT
       ACATTTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAATA

22951  TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT
       AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001  TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG
       AATTTTTAGT TTCCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051  ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAAACTC AGGCACAACC
       TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101  ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC
       TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151  CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC
       GTTGCGCAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201  CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC
       GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251  ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT
       TGATAGTCGC GGCCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301  CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT
       GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351  TTGGTAGCTG CCTTCCCAAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC
       AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401  TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG
       AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451  ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT
       TATGTCGCGG ACGTATTTTC GGAACTAGAC GAATTTTCGG TGGACTCGGA

23501  TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAACTGATTG
       AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551  GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTGGAGAT
       CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601  CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG
       GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651  ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA
       TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT
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Figure 26 Y

23701 ATCACGTGCT C[redacted]TATTTAT CATAATGCTT CCGTGTAGAC ACTTAA[redacted]C
TAGTGCACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT
CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGCGCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG
GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG
TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG
GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC
GAAGGTGAAC CAGTCCGTCA TCAAACTTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA
TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT
GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCCTCTT GCGTCCGCAT ACCACGCGCC
GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGCGCGG

24151 ACTGGGTCGT CTTCATTCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC
TGACCCAGCA GAAGTAAGTC GGCGGCGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTTGCTGAA ACCCACCATT TGTAGCGCCA
TACGAACTAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG
GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC
GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTCG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA
GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCCTC
CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GGCGGCGACG GGGACGGGGA
TAGGCGAAAA AACCCCCGCG GGCCCCTCCG CCGCCGCTGC CCCTGCCCCT

24501 CGACACGTCC TCCATGGTTG GGGGACGTCG CGCCGCACCG CGTCCGCGCT
GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC
GCCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC
ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

Figure 26Z

24651 CGCCCCCTCT G[illegible]TCGCCA CCACCGCCTC CACCGATGCC GCCAAC[illegible]C
GCGGGGGAGA C[illegible]AAGCGGT GGTGGCGGAG GTGGCTACGG CGGTTG[illegible]CG

24701 CTACCACCTT CCCCGTCGAG GCACCCCCGC TTGAGGAGGA GGAAGTGATT
GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA

24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT
TAGCTCGTCC TGGGTCCAAA ACATTCGCTT CTGCTGCTCC TGGCGAGTCA

24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG
TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCGTCTC CGTTTGCTCC

24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA
TTGTTCAGCC CGCCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCCT

24901 GACGACGTGC TGTTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA
CTGCTGCACG ACAACTTCGT AGACGTCGCG GTCACGCGGT AATAGACGCT

24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCCT CGCCATAGCG GATGTCAGCC
GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG

25001 TTGCCTACGA ACGCCACCTA TTCTCACCGC GCGTACCCCC CAAACGCCAA
AACGGATGCT TGCGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT

25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCCGTATT
CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACTGCA
ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT

25151 AGATACCCCT ATCCTGCCGT GCCAACCGCA GCCGAGCGGA CAAGCAGCTG
TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC

25201 GCCTTGCGGC AGGGCGCTGT CATACCTGAT ATCGCCTCGC TCAACGAAGT
CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA

25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG
CGGTTTTTAG AAACTCCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACTCTGG AGTGTTGGTG
GAGACGTTGT CCTTTTGTCG CTTTTACTTT CAGTGAGACC TCACAACCAC

25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA
CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

25401 GGTCACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCCC AAGGTCATGA
CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT

25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG
CGTGTCAGTA CTCACTCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA
CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT

25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG
GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26AA

25601 AGCGACGCAA A[illegible]AATGATG GCCGCAGTGC TCGTTACCGT GGAGCT[illegible]AG
TCGCTGCGTT TGATTACTAC CGGCGTCACG AGCAATGGCA CCTCGAACTC

25651 TGCATGCAGC GGTTCTTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA
ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT

25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA
TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC
AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAAACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC
CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT
CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC
CCGTCTGCCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC
TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GGCGGACATC ATTTTCCCCG
GAAGTTGCTC GCGAGGCACC GGCGCGTGGA CCGCCTGTAG TAAAAGGGGC

26051 AACGCCTGCT TAAAACCCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA
TTGCGGACGA ATTTTGGGAC GTTGTCCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT
TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA

26151 GCCCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC
CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC
CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG
TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGTCACTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC
AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT
ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTTGAA
CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCCAACTT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG
TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501 AGGACTACCA CGCCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG
TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG A[illegible]TTACCGC CTGCGTCATT ACCCAGGGCC ACATTC[illegible]G
GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC

26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG
GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC

26651 GACGGGGGGT TTACTTGGAC CCCCAGTCCG GCGAGGAGCT CAACCCAATC
CTGCCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG

26701 CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA
GGGGGCGGCG GCGTCGGGAT AGTCGTCGTC GGCGCCCGGG AACGAAGGGT

26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG
CCTACCGTGG GTTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC

26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA
CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT

26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCG
CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC

26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CGGTCGCATT CCCCTCGCCG
TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC

26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC
CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG

27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA
AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCCTGT

27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA
GGTGACCTTG GTCCCGGCCA TTCAGGTTCG TCGGCGGCGG CAATCGGGTT

27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC
CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG TGTTCTTGCG

27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCGCC
GTATCAACGA ACGAACGTTC TGACACCCCC GTTGTAGAGG AAGCGGGCGG

27201 GCTTTCTTCT CTACCATCAC GGCGTGGCCT TCCCCCGTAA CATCCTGCAT
CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA

27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA
ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT

27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA
GTCGTCGCCG GTGTGTCTTC GTTTCCGCTG GCCTATCGTT CTGAGACTGT

27351 AAGCCCAAGA AATCCACAGC GGCGGCAGCA GCAGGAGGAG GAGCGCTGCG
TTCGGGTTCT TTAGGTGTCG CCGCCGTCGT CGTCCTCCTC CTCGCGACGC

27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT
AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCCTAAA

27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG
AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTTCTC

Figure 26 AC

27501 CTGAAAATAA [redacted]CAGGTC TCTGCGATCC CTCACCCGCA GCTGC[redacted]A
GACTTTTATT TTTTGTCCAG AGACGCTAGG GAGTGGGCGT CGACGGACAT

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC
AGTGTTTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT
AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC
AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG

27701 GCCAGCACCT GTTGTCAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT
CGGTCGTGGA CAACAGTCGC GGTAATACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA
TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC
CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG
GGCCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTCC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC
GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC
CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG
GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTCGC CCGGGCAGGG TATAACTCAC
GCCCGCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC
GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GGCGCCGGCC
GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC
CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAACT CTGCAATTTA TTGAGGAGTT
AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC
ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC
GCCTAGTTAA ATAAGGATTG AAACTGCGCC ATTTCCTGAG CCGCCTGCCG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT
ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

```
28451  GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT
       CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCGCTGAGG CCACTCAAAA

28501  GCTACTTTGA ATTGCCCGAG GATCATATCG AGGGCCCGGC GCACGGCGTC
       CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG

28551  CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC
       GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601  CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG
       GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651  TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT
       ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

28701  CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT
       GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751  ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG
       TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC

28801  CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG
       GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851  TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT
       AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901  ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT
       TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951  GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT
       CGCAGTGGCC GGCGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001  TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT
       AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA

29051  AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT
       TCTTTTGGGA ATCCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101  GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA
       CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT

29151  TCGGGGTTGG GGTTATTCTC TGTCTTGTGA TTCTCTTTAT TCTTATACTA
       AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201  ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA
       TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251  TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT
       AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA

29301  AATCCTAGGT TTACTCACCC TTGCGTCAGC CCACGGTACC ACCCAAAAGG
       TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

29351  TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT
       ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA
```

Figure 26 AE

29401 GAGTGCACCA CTCTTATAAA ATGCACCACA GAACATGAAA AGCTGCTTAT
CTCACGTGGT GAGAATATTT TACGTGGTGT CTTGTACTTT TCGACGAATA

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC
AGCGGTGTTT TTGTTTTAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT
GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT
TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA
CATGTACTCG TTTGTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG
TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA
CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCACCACT
CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA
TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCCGGTCAT TTCCTGCTCA ATACCATTCC
TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA
GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACTT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC
CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTCGT GGACAGGGCG

30001 GGATTTGTTC CAGTCCAACT ACAGCGACCC ACCCTAACAG AGATGACCAA
CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC
GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACTTGGG CATGTGGTGG
GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG
AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG
GACGGATTTC GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC
ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT
TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30351 TTTATATTAC T[illegible]CCTTGT TGCGCTTTTT TGTGCGTGCT CCACAT[illegible]C
AAATATAATG ACTGGGAACA ACGCGAAAAA ACACGCACGA GGTGTAACCG

30401 TGCGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT
ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG
ACGAAATGCC TAAACAGTGG GAGTGCGAGT AGACGTCGGA GTAGTGACAC

30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA
CAGTAGCGGA AATAGGTCAC GTAACTGACC CAGACACACG CGAAACGTAT

30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA
AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT

30601 GAATTCTTTA ATTATGAAAT TTACTGTGAC TTTTCTGCTG ATTATTTGCA
CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

30651 CCCTATCTGC GTTTTGTTCC CCGACCTCCA AGCCTCAAAG ACATATATCA
GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG
ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTTCT
GCTAGAAAGG CTTCGGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG
CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAACT GTAACCGACC

30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT
TTGCGTTATC TACGGTACTT GGTGGGTTGA AAGGGGCGCG GGCGATACGA

30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC
AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

30951 GCCCACCTTC TCCCACCCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA
CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT

31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC
CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG

31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT
TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CGCGTACTTA

31101 CAAGAGCTCC AAGACATGGT TAACTTGCAC CAGTGCAAAA GGGGTATCTT
GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA

31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC
AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG

31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG
TGGCGGAATC GATGTTCAAC GGTTGGTTCG CAGTCTTTAA CCACCAGTAC

31251 GTGGGAGAAA AGCCCATTAC CATAACTCAG CACTCGGTAG AAACCGAAGG
CACCCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31301 CTGCATTCAC TCACCTTGTC AAGGACCTGA GGATCTCTGC ACCCTTATTA
GACGTAAGTG AGTGGAACAG TTCCTGGACT CCTAGAGACG TGGGAATAAT

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT
TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC
GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG
CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACTGT GCCTTTTCTT ACTCCTCCCT TTGTATCCCC
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTTA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT
GAGAGAGACC TGCTCCGGCC GTTGGAATGG AGGGTTTTAC ATTGGTGACA

31851 GAGCCCACCT CTCAAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG
CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT
GTGGGGAGTG TCAATGGAGT CTTCGGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCGCTAAC
GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

32001 CGTGCACGAC TCCAAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT
GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGGCTA

32101 AGCAGTACCC TTACTATCAC TGCCTCACCC CCTCTAACTA CTGCCACTGG
TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC
ATCGAACCCG TAACTGAACT TTCTCGGGTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT
ATCCTGATTT CATGCCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH

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32251  TTGACCGTAG G[illegible]CTGGTCC AGGTGTGACT ATTAATAATA CTTCCT[illegible]CA
       AACTGGCATC C[illegible]GACCAGG TCCACACTGA TAATTATTAT GAAGGA[illegible]GT

32301  AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC
       TTGATTTCAA TGACCTCGGA ACCCAAAACT AAGTGTTCCG TTATACGTTG

32351  TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA
       AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTTGTC TGCGGAATAT

32401  CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT
       GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451  AGGACAGGGC CCTCTTTTTA TAAACTCAGC CCACAACTTG GATATTAACT
       TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA

32501  ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT
       TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551  GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT
       CTCCAATTGG ATTCGTGACG GTTCCCCAAC TACAAACTGC GATGTCGGTA

32601  AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA
       TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651  ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA
       TGTGTTTAGG GGAGTTTTGT TTTTAACCGG TACCGGATCT TAAACTAAGT

32701  AACAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC
       TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG

32751  AGGTGCCATT ACAGTAGGAA ACAAAAATAA TGATAAGCTA ACTTTGTGGA
       TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801  CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT
       GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851  AAACTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT
       TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901  TTCAGTTTTG GCTGTTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC
       AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CCTTGTCAAG

32951  AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC
       TTTCACGAGT AGAATAATAT TCTAAACTGC TTTTACCTCA CGATGATTTG

33001  AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC
       TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051  TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG
       ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101  CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA
       GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCATTGTA ACAGTCAGTT

33151  GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT
       CAAATGAATT TGCCTCTGTT TTGATTTGGA CATTGTGATT GGTAATGTGA
```

Figure 26 AI

33201 AAACGGTACA CAGGAAACAG GAGACACAAC TCCAAGTGCA TACTCT[illegible]T
TTTGCCATGT GTCCTTTGTC CTCTGTGTTG AGGTTCACGT ATGAGA[illegible]CA

33251 CATTTTCATG GGACTGGTCT GGCCACAACT ACATTAATGA AATATTTGCC
GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAATTACT TTATAAACGG

33301 ACATCCTCTT ACACTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG
TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGTTATGTTT CAACGTGTTT ATTTTTCAAT TGCAGAAAAT TTCAAGTCAT
ACAATACAAA GTTGCACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT
CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC
GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA
AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT
CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA
CCCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AAACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC
CGCGCGCTTA TTTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCCGCA GCATAAGGCG
TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCCT GATCTCACTT AAATCAGCAC
GGAACAGGAG GCCCGTGTCG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG
TCATTGACGT CGTGTCGTGG TGTTATAACA AGTTTTAGGG TGTCACGTTC

34001 GCGCTGTATC CAAAGCTCAT GGCGGGGACC ACAGAACCCA CGTGGCCATC
CGCGACATAG GTTTCGAGTA CCGCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG
TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC
TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34151 CATATAAACC T[illegible]GATTAAA CATGGCGCCA TCCACCACCA TCCTAA[illegible]CA
GTATATTTGG A[illegible]CTAATTT GTACCGCGGT AGGTGGTGGT AGGATT[illegible]GT

34201 GCTGGCCAAA ACCTGCCCGC CGGCTATACA CTGCAGGGAA CCGGGACTGG
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT
CAGTACTATA GTTACAACCG TGTTGTGTCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTTGGG

34401 ATTCCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTTGG TCGTAGTGTC
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCCTGAAG CAAAACCAGG
TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC
ACGCCCGCAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGCTTCG GGTTCTATGT AAACTCCTTC ATGCGCCGCT GCCCTGATAA
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCGTTC
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG
AAAAAATAAG GTTTTCTAAT AGGTTTTGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GGCGTGGTCA AACTCTACAG CCAAAGAACA
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTTCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGCACAAT GGCTTCCAAA AGGCAAACGG
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC
GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26AK

35101 TCTATAAACA T[illegible]AGCACC TTCAACCATG CCCAAATAAT TCTCAT[illegible]G
AGATATTTGT AA[illegible]GTCGTGG AAGTTGGTAC GGGTTTATTA AGAGTA[illegible]GC

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA
GGTGGAAGAG TTATATAGAG ATTCGTTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA
AACATTTTTA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA
TAGTACTAAC GTTTTTAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC
TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC
GCGGTCCTTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TGCGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA
GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTCG

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAAACA TTTAAACATT
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA
TCTTCGGACA GAATGTTGTC CTTTTTGTTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCATGTCC GGAGTCATAA TGTAAGACTC
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA
CCATTTGTGT AGTCCAACTA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTTGT GTATTTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA
ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36051 CATACAGCGC TTCCACAGCG GCAGCCATAA CAGTCAGCCT TACCAGTAAA
      GTATGTCGCG AAGGTGTCGC CGTCGGTATT GTCAGTCGGA ATGGTCATTT

36101 AAAGAAAACC TATTAAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA
      TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT

36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA
      CAGTGTCACA TTTTTTCCCG GTTCACGTCT CGCTCATATA TATCCTGATT

36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG
      TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC

36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT
      GCTTGGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTTG AAGGAGTTTA

36301 CGTCACTTCC GTTTTCCCAC GTTACGTCAC TTCCCATTTT AAGAAAACTA
      GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT

36351 CAATTCCCAA CACATACAAG TTACTCCGCC CTAAAACCTA CGTCACCCGC
      GTTAAGGGTT GTGTATGTTC AATGAGGCGG GATTTTGGAT GCAGTGGGCG

36401 CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA
      GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT

PacI

36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA
      ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT

36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC
      TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG

36551 TCGCTTCCGG CGGCATCGGG ATGCCCGCGT TGCAGGCCAT GCTGTCCAGG
      AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC

36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG
      GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC

36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
      CTTGGCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG

36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG
      GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC

36751 ACAGGACTAT AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG
      TGTCCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC

36801 CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
      GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG

36851 CTTCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT
      GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA

36901 TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT
      AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

36951 TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC
AGTCGGGCTG GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGGTTGG

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC
TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA
TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT

37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG
TGGTGGCGAC CATCGCCACC AAAAAAACAA ACGTTCGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG
GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT
AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT
CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG
GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA
ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG
GGTCGGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG
GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT
GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC
GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT
AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26AN

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37901  CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCGGTAA
       GTACCAATAC CGTCGTGACG TATTAAGAGA ATGACAGTAC GGTAGGCATT

37951  GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
       CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001  TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC
       ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051  CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
       GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101  CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG
       GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151  TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
       ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201  CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
       GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTTCCCTT

38251  TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
       ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301  TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA
       ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351  ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
       TACATAAATC TTTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401  AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT
       TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451  AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA
       TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

                    PacI
                  ~~~~~~~
38501  TTCTTAATTT CTTAATTAA  (SEQ ID NO:32)
       AAGAATTAAA GAATTAATT  (SEQ ID NO:33)
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Figure 26 AO

MRKAd5nef MER1063

(MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

```
  1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
    GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

 51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
    CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
    ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
    CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
    CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA
    GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
    TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
    CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
    GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
    CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
    ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
    ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
    ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
    GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
    TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
    TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
    GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT
```

Figure 27A

```
851  CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
     GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

901  TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
     AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951  TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
     ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
     ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
     TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
     CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
     GGTAGGTGCG ACAAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
     AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCGGCT
     CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
     CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
     CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
     GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
     GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
     CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
     GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
     GGGTCTTCTC CGTCCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC
     ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG
     GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAACT GCGCCGCCCA CCCCATGTCC
     ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG
```

Figure 27B

```
1801  CAGCACGGCA TCGAGGACCC CGAGAAGGAG GTGCTGGAGT GGAGGTTCGA
      GTCGTGCCGT AGCTCCTGGG GCTCTTCCTC CACGACCTCA CCTCCAAGCT

1851  CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
      GAGGTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901  ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
      TGTTCCTGAC GATTTCGGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951  CCATCTGTTG TTTGCCCCTC CCCCGTGCCT TCCTTGACCC TGGAAGGTGC
      GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001  CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC
      GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051  TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
      ACTCATCCAC AGTAAGATAA GACCCCCCAC CCCACCCCGT CCTGTCGTTC

2101  GGGGAGGATT GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC
      CCCCTCCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151  TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
      ATACCGGCTA GCCGCGCGGC ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201  GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTTGT ATCTGTTTTG
      CCCTTTCTTA TATATTCCAC CCCCAGAATA CATCAAAACA TAGACAAAAC

2251  CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG
      GTCGTCGGCG GCGGCGGTAC TCGTGGTTGA GCAAACTACC TTCGTAACAC

2301  AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TGCGTCAGAA
      TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351  TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACTCTA
      ACACTACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401  CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC
      GATGGAACTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451  TCCGCCGCCG CTTCAGCCGC TGCAGCCACC GCCCGCGGGA TTGTGACTGA
      AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501  CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG
      GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551  CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
      GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAAACTGG

2601  CGGGAACTTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
      GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651  TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA
      AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701  AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT
      TTTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA
```

Figure 27C

```
2751  TATTTAGGGG TTTTGCGCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC
      ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801  GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGACTCTGGA
      CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTCC ACTGAGACCT

2851  TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
      ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901  TGCAGAGCTT CATGCTGCGG GGTGGTGTTG TAGATGATCC AGTCGTAGCA
      ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951  GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG
      CCTCGCGACC CGCACCACGG ATTTTTACAG AAAGTCATCG TTCGACTAAC

3001  CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT
      GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051  GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTTAGGTT
      CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101  GGCTATGTTC CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA
      CCGATACAAG GGTCGGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151  CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA
      GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201  GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
      CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251  CATGCATTCG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG
      GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCGC CGCCGGACCC

3301  CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTC CAGGATGAGA
      GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351  TCGTCATAGG CCATTTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
      AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCCACG GTCTGACGCC

3401  TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA
      ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451  TTTCCCACGC TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG
      AAAGGGTGCG AAACTCAAGT CTACCCCCCT AGTACAGATG GACGCCCCGC

3501  ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG
      TACTTCTTTT GCCAAAGGCC CCATCCCCTC TAGTCGACCC TTCTTTCGTC

3551  GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCG TAAATCACAC
      CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601  CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCATCC
      GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651  CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTC
      GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG
```

Figure 27 D

3701 CCTGACCAAA T[illegible]GCCAGAA GGCGCTCGCC GCCCAGCGAT AGCAGT[illegible]TT
GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG
CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC
GAAAACTCGC AAACTGGTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
GACGAGATGC CGTAGAGCTA GGTCGTATAG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG
CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG
AGTACAGAAA GGTGCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT
CACTTCCCCA CGCGAGGCCC GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GGTAGCATTT GACCATGGTG TCATAGTCCA GCCCCTCCGC GGCGTGGCCC
CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
AACCGCGCGT CGAACGGGAA CCTCCTCCGC GGCGTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
TGAAAACTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
TCCGTAGGCG CGGCGTCCGG GGCGTCTGCC AGAGCGTAAG GTGCTCGGTC

4301 GTGAGCTCTG GCCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT
CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA
CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCCTCG
GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACTCTCC GGACAGGAGC

4451 AGCGGTGTTC CGCGGTCCTC CTCGTATAGA AACTCGGACC ACTCTGAGAC
TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTCACC CTCCCCATCG

4551 GGTCGTTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCCACAC TTCTGTGTAC

4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTTTGTAGG TGTAGGCCAC
AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4651 GTGACCGGGT [redacted]CCTGAAG GGGGGCTATA AAAGGGGGTG GGGGC[redacted]TT
CACTGGCCCA CAAGGACTTC CCCCCGATAT TTTCCCCCAC CCCCGCGCAA

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGCG GTGATGCCTT
AAGGTTTTTG CTCCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA
ACTCCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTCGCGATC GGCGCGCTCC TTGGCCGCGA
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGGCGCT

5001 TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051 GTGGTGCGCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTTGTGCAG
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT
AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCAG
TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTTCT GGGGCCCGTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTC AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCCT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA
AAGGTGGCGC CTACGACCGC GCGTGCATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5601 GGAAGACGTT G[illegible]CTGGCG TCTGTGAGAC CTACCGCGTC ACGCAG[illegible]G
CCTTCTGCAA CTTCGACCGC AGACACTCTG GATGGCGCAG TGCGTCTTC

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC
CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT
CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAAACTC TTCGCGGTCT
CAGGGAAAAA AAAGGTGTCG AGCGCCAACT CCTGTTTGAG AAGCGCCAGA

5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC
AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA
ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC
GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT
CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA

6001 GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTTGG
CAGCAGCGTA GGCGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC
TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA
CGCGCTCCGT ATTTCAACGC ACACTACGCC TTCCCAGGGC CGTGGAGCCT

6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA
TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201 TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG
ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACTAC

6251 GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG
CTTCCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA
GGGCACGAGA CTTTCCCGGG TCAGACGTTC TACTCCCAAC CTTCGCTGCT

6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG
TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401 GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA
CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451 GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTC GCGGCTAGGT
CCATTCGCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACTT CATGACCAGC
GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG

Figure 27G

6551 ATGAAGGGCA C[illegible]CTGCTT CCCAAAGGCC CCCATCCAAG TATAG[illegible]C
TACTTCCCGT GCTCGACGAA GGGTTTCCGG GGGTAGGTTC ATATCCAGAG

6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG
ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC

6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG
CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACCGA TAACTACACC

6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA
ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT

6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA
TTTTGCACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT

6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC
CCAACTGGAC TGCTGGCGCG TGTTCCTTCG TCTCACCCTT AAACTCGGGG

6851 TCGCCTGGCG GGTTTGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG
AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC

6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC
TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTGG TGGTGCGGCG

6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTCGGAG CTTGATGACA
CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT

7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG
TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC

7051 GTCAGGCGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC
CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG

7101 GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGCG
CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCGACCAA CCACCGCCGC

7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GGCGCGACTA CGGTACCGCG
AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC

7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCCTT GGATGATGCA TCTAAAAGCG
GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC

7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA
CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCCT GGGCGGCCCT

7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC
CTCCCCCGTC CCCGTGCAGC CGCGGCGCGC GCCCGTCCTC GACCACGACG

7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTTGA TCTCCTGAAT
CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA

7401 CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG
GACCGCGGAG ACGCACTTCT GCTGCCCGGG CCACTCGAAC TTGGACTTTC

7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAAA
TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCCG GACCGCGTTT

Figure 27H

7501 ATCTCCTGCA C[redacted]CTCCTGA GTTGTCTTGA TAGGCGATCT CGGC[redacted]A
TAGAGGACGT G[redacted]GAGGACT CAACAGAACT ATCCGCTAGA GCCGGT[redacted]T

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG
GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCGGCATC
TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA
CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG
TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA
CAACTATAGG GGGTTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GGCGACAGTG TCGCGCACCT CGCGCTCAAA
AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT
CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA

8051 CCCCTTCTTC TTCTTCTGGC GGCGGTGGGG GAGGGGGGAC ACGGCGGCGA
GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCCTG TGCCGCCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA
CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT

8201 GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG
CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCCGAC

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT
GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
TCCATGAGGC GGCGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG
TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCGTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA
TCGTGGCACC GCCCGCCGTC GCCCGCCGCC AGCCCCAACA AAGACCGCCT

Figure 27I

8451 GGTGCTGCTG A[illegible]TGTAAT TAAAGTAGGC GGTCTTGAGA CGGCGG[illegible]GG
CCACGACGAC TACTACATTA ATTTCATCCG CCAGAACTCT GCCGCCTACC

8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG
AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC

8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA
AGCCGGTACG GGGTCCGAAG CAAAACTGTA GCCGCGTCCA GAAACATCAT

8601 GTCTTGCATG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTTGTC
CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG

8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG
GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC

8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG
ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC

8751 AAGCAGGGCT AGGTCGGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA
TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT

8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT
GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA

8851 GCGCCCGTGT TGATGGTGTA AGTGCAGTTG GCCATAACGG ACCAGTTAAC
CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG

8901 GGTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG
CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC

8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT
GGGAGCTCAG TTTATGCATC AGCAACGTTC AGGCGTGGTC CATGACCATA

9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT
GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA

9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT
CCGGCCCCGA GGCCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA

9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC
TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG

9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC
CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG

9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT
GTACCAGCCC TGCGAGACCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA

9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT
TCTGGCACGT TTTCCTCTCG GACATTCGCC CGTGAGAAGG CACCAGACCA

9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT
CCTATTTAAG CGTTCCCATA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA

9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTCGAACCC
TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9401 AGGTGTGCGA C[illegible]AGACAA CGGGGGAGTG CTCCTTTTGG CTTCCT[illegible]A
TCCACACGCT GCAGTCTGTT GCCCCCTCAC GAGGAAAACC GAAGGAAGGT

9451 GGCGCGGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG
CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG CGCGCGTCGC

9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG
ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551 CCGGAGGGTT ATTTTCCAAG GGTTGAGTCG CGGGACCCCC GGTTCGAGTC
GGCCTCCCAA TAAAAGGTTC CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA
AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT
CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAAACGA

9701 TTTCCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCCC TCCTCAGCAG
AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCT CCCCTCCTCC
GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG
TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901 GGCGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG
CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTC

9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG
ACAAAGCGCT GGCGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GGCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT
AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCGGGATT AGTCCCGCGC
CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GGCGGCCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG
CGCGTGTGCA CCGCCGGCGG CTGGACCATT GGCGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAACTTTCA AAAAAGCTTT AACAACCACG TGCGTACGCT
TTGGTCCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG
ATTCGCGCGA CCTCGTTTTG GGTTTATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

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10351  TTCCTTATAG T[illegible]GCACAG CAGGGACAAC GAGGCATTCA GGGATG[illegible]T
       AAGGAATATC ACGTCGTGTC GTCCCTGTTG CTCCGTAAGT CCCTACGCGA

10401  GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA
       CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT

10451  TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG
       AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501  GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCG
       CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC

10551  CAAGATATAC CATACCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG
       GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCCTC CATTTCTAGC

10601  AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC
       TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG

10651  CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG
       GACCCGCAAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701  GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC
       CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCCGGG

10751  TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG
       ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801  GGCGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG
       CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC

10851  GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG
       CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCGC

10901  GCGTGGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG
       CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951  TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG
       ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC

11001  GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA
       CGCCACGCCC GCCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051  CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC
       GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101  CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG
       GACTGCGCAA GGCCGTCGTC GGCGTCCGGT TGGCCGAGAG GCGTTAAGAC

11151  GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC
       CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TGCGTGCTCT TCCACGACCG

11201  GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG
       CTAGCATTTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC

11251  GCCTGGTCTA CGACGCGCTG CTTCAGCGCG TGGCTCGTTA CAACAGCGGC
       CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCCG
```

Figure 27L

11301 AACGTGCAGA CCAACCTGGA CCGGCTGGTG GGGGATGTGC GCGAGGCCGT
TTGCACGTCT GGTTGGACCT GGCCGACCAC CCCCTACACG CGCTCCGGCA

11351 GGCGCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG
CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC

11401 CACTAAACGC CTTCCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG
GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTTGCACGG CGCCCCTGTC

11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGACTGAGAC
CTCCTGATGT GGTTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG

11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA
TGGCGTTTCA CTCCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT

11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAAACTTG
CATCTGTTCC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTTGAAC

11601 CAGGGGCTGT GGGGGGTGCG GGCTCCCACA GGCGACCGCG CGACCGTGTC
GTCCCCGACA CCCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG

11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT
ATCGAACGAC TGCGGGTTGA GCGCGGACAA CGACGACGAT TATCGCGGGA

11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACCTAGG TCACTTGCTG
AGTGCCTGTC ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC

11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT
TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA

11801 CCAGGAGATT ACAAGTGTCA GCCGCGCGCT GGGGCAGGAG GACACGGGCA
GGTCCTCTAA TGTTCACAGT CGGCGCGCGA CCCCGTCCTC CTGTGCCCGT

11851 GCCTGGAGGC AACCCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC
CGGACCTCCG TTGGGATTTG ATGGACGACT GGTTGGCCGC CGTCTTCTAG

11901 CCCTCGTTGC ACAGTTTAAA CAGCGAGGAG GAGCGCATTT TGCGCTACGT
GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA

11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCCAGCG
CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TGCGGGTCGC

12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA
ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATACGGAGT

12051 AACCGGCCGT TTATCAACCG CCTAATGGAC TACTTGCATC GCGCGGCCGC
TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG

12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC
GCACTTGGGG CTCATAAAGT GGTTACGGTA GAACTTGGGC GTGACCGATG

12151 CGCCCCCTGG TTTCTACACC GGGGGATTCG AGGTGCCCGA GGGTAACGAT
GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA

12201 GGATTCCTCT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA
CCTAAGGAGA CCCTGCTGTA TCTGCTGTCG CACAAAAGGG GCGTTGGCGT

Figure 27 M

12251 GACCCTGCTA G[illegible]TTGCAAC AGCGCGAGCA GGCAGAGGCG GCGCTG[illegible]A
CTGGGACGAT C[illegible]AACGTTG TCGCGCTCGT CCGTCTCCGC CGCGAC[illegible]T

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
TCCTTTCGAA GGCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTTCCA AGCTTGATAG GGTCTCTTAC
GGCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA
GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATTT
TGTTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC
CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTTGGGA GGGAGTGGCA ACCCGTTTGC
CGTCTGCTGT CGTCGCAGGA CCTAAACCCT CCCTCACCGT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAAAAAAAA AAAAAGCATG
CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAACTCAC CAAGGCCATG GCACCGAGCG TTGGTTTTCT
TACGTTTTAT TTTTTGAGTG GTTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC
ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTCACCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC
CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC
GACGCCGGAT GGCCCCCCTC TTTGTCGTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTC AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
AGAACTGCTG GCCAGCGTGA CCCCGCCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13201 CCAACATGCC AAAGGTGAAC GAGTTCATGT TTACCAATAA GTTTAAGCTG
GGTTGTACGG TTTCCACTTG CTCAAGTACA AATGGTTATT CAAATTCGAC

13251 CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA
GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT

13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCGA GGGCAACTAC TCCGAGACCA
TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT

13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG
ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTCAC

13401 GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC
CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACTGTG

13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG
GGCGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC

13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA
CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAGTAAAA CGACGGTCCT

13551 TGCGGGGTGG ACTTCACCCA CAGCCGCCTG AGCAACTTGT TGGGCATCCG
ACGCCCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC

13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG
GTTCGCCGTT GGGAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC

13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC
TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG

13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG
AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC

13751 CAGTGGCAGC GGCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC
GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG

13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC
TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG

13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC
TGTGCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG

13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA
GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT

13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC
AGTTTGGGGA CTGTCTCCTG TCGTTCTTTG CGTCAATGTT GGATTATTCG

14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA
TTACTGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT

14051 CGGCGACCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACTCCTG
GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC

14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTCGTTGCC AGACATGATG
TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

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14151  CAAGACCCCG T[redacted]TTCCG CTCCACGCGC CAGATCAGCA ACTTTC[redacted]T
       GTTCTGGGGC ACTGGAAGGC GAGGTGCGCG GTCTAGTCGT TGAAAGGCCA

14201  GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC
       CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251  AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG
       TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301  TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAC
       AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG GTCGGGGGTG

14351  CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC
       GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTGCCCTGCG

14401  TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC
       ATGGCGACGC GTTGTCGTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451  GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC
       CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501  GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA
       CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551  TATCGCCCAG CAATAACACA GGCTGGGGCC TGCGCTTCCC AAGCAAGATG
       ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601  TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG
       AAACCGCCCC GGTTCTTCGC GAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651  GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA
       CGTGATGGCG CGCGGGACCC CGCGCGTGTT TGCGCCGGCG TGACCCGCGT

14701  CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC
       GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751  ACGCCCACGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT
       TGCGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA

14801  GGTGCGCGGA GCCCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG
       CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851  TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG
       ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901  GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG
       CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951  GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA
       CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001  GGCGACGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG
       CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAATCACG ATACTGAGTC

15051  GGTCGCAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG
       CCAGCGTCCC CGTTGCACAT AACCCACGCG CTGAGCCAAT CGCCGGACGC
```

Figure 27P

```
15101 CGTGCCCGTG C[redacted]CCCGCC CCCCGCGCAA CTAGATTGCA AGAAAA[redacted]T
      GCACGGGCAC G[redacted]GGGCGG GGGGCGCGTT GATCTAACGT TCTTTT[redacted]A

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA
      TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC
      CGATACAGGT TCGCGTTTTA GTTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA
      CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAACTTGAC
      TCGATTTCGC CCAGTTTTTC TTTTTCTTTC TACTACTACT ACTTGAACTG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG
      CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC

15401 GAAAGGTCGA CGCGTAAAAC GTGTTTTGCG ACCCGGCACC ACCGTAGTCT
      CTTTCCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCGG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG
      AATGCGGGCC ACTCGCGAGG TGGGCGTGGA TGTTCGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA
      CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCCT

15551 GTTTGCCTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG
      CAAACGGATG CCTTTCGCCG TATTCCTGTA CGACCGCAAC GGCGACCTGC

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG
      TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCCTAAAGC GCGAGTCTGG
      GGGCGCGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCG CGCTCAGACC

15701 TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG
      ACTGAACCGT GGGTGGCACG TCGACTACCA TGGGTTCGCG GTCGCTGACC

15751 AAGATGTCTT GGAAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC
      TTCTACAGAA CCTTTTTTAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGACTGGGCG TGCAGACCGT
      GCGCACGCCG GTTAGTTCGT CCACCGCGGC CCTGACCCGC ACGTCTGGCA

15851 GGACGTTCAG ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG
      CCTGCAAGTC TATGGGTGAT GGTCATCGTG GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGGTTG CCTCAGCGGT GGCGGATGCC
      TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCCTACGG

15951 GCGGTGCAGG CGGTCGCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA
      CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGGCGC CCGCGCCGTT
      TTGCCTGGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GGCGCGGCAA
```

Figure 27Q

16051 CGAGGAAGTA C[redacted]GCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTA[redacted]T
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT
CAGCGGCAGC GGTCGGGCAC GACCGGGGCT AAAGGCACGC GTCCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCCAG
GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA
TCCCCGTACC GGCCGGTGCC GGACTGCCCG CCGTACGCAG CACGCGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTCGCAT GCGCGGCGGT ATCCTGCCCC
GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA CGCGCCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA
AGGAATAAGG TGACTAGCGG CGCCGCTAAC CGCGGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAAAAACAA GTTGCATGTG
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTTGTT CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCCTGTAA
CTTTTTAGTT TTATTTTTCA GACCTGAGAG TGCGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCCGCGACA
GATAAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTTA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGA CCTGGCCAAC
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17001 AGAGGAGCCT C[redacted]CGGCCG TGGAGACAGT GTCTCCAGAG GGGCGT[redacted]G
TCTCCTCGGA GG[redacted]GGCCGGC ACCTCTGTCA CAGAGGTCTC CCCGCAC[redacted]GC

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC
TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCACTAAAG CAAGGCCTGC CCACCACCCG
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGGC

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA
AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCGACCG CCGTTGTTGT AACCCGTCCT AGCCGCGCGT CCCTGCGCCG
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGGC

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC
GCGGCGGTCG CCAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC
TTTCGTGTGA CTTGTCGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GGCGCGCGGG CGAAAGGTTC

17501 ATGGCTACCC CTTCGATGAT GCCGCAGTGG TCTTACATGC ACATCTCGGG
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGCAG TTTGCCCGCG
GGTCCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAA TAAACCTAGA AGAAGAGGAC
TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

Figure 27S

17951 GATGACAACG A[redacted]CGAAGT AGACGAGCAA GCTGAGCAGC AAAAAA[redacted]A
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTTTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTCGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAA

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT
AGTACGTCGA CCCTCTCAGG ATTTTTTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTCATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT
TGCCAAGTAT ACGTTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTTT
CATTTCGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCCAGA CACTCATATT
ACCATAACAT GTCACTTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTTGTAGAT TTGCAAGACA GAAACACAGA
GTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAACTGA AGATGAACTT CCAAATTACT GCTTTCCACT
TAACTTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAACAG
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAT
CAGTCCTTTT ACCTACCCTT TTTCTACGAT GTCTTAAAAG TCTATTTTTA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18901 CCTGTGGAGA A[illegible]TCCTGT ACTCCAACAT AGCGCTGTAT TTGCCC[illegible]A
GGACACCTCT TTAAAGGACA TGAGGTTGTA TCGCGACATA AACGGGCTGT

18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC
TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA
ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT

19051 CATTAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC
GTAATTGGAA CCTCGTGCGA CCAGGGAACT GATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTTAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC
GATTCCCAAC TGCCTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC
GTGGAAGAAG GGGTACCGGG TGTTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC
AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA
GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC
TCTGATTCCT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA
GAAATTCTTC CACCGGTAAT GGAAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTCGC GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCCT
CCCCTCCCAA TGTTGCAACG GGTCACATTG TACTGGTTTC TGACCAAGGA

19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC
CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 27 U

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19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC
      GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT
      TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC
      CCCGTAGGAT GTGGTTGTGT TGTTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACTTCCC CTATCCGCTT
      GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA
      TATCCGTTCT GGCGTCAACT GTCGTAATGG GTCTTTTTCA AAGAAACGCT

20101 TCGCACCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG
      AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCCACGCG
      GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TGCGGTTGAG GCGGGTGCGC

20201 CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA
      GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG
      ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GGCGTGGCGC

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CCTTCTCGGC CGGCAACGCC
      CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC
      TGTTGTATTT CTTCGTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAAA GATCTTGGTT GTGGGCCATA
      TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA
      AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA
      TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT
      GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG
      ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCGACCGC
      TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC
      ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC
      GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG
```

Figure 27V.

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20801  CCCAAACTCC C[illegible]GATCAC AACCCCACCA TGAACCTTAT TACCGG[illegible]A
       GGGTTTGAGG GTACCTAGTG TTGGGGTGGT ACTTGGAATA ATGGCCCCAT

20851  CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TGCGTCGCAA
       GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901  CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA
       GGTCCTTGTC GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951  GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTTGTCA CTTGAAAAAC
       CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001  ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA
       TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAT

21051  TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG
       AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC

21101  TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG
       AAATTTTTAG TTTCCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151  GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC
       CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201  CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA
       GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251  CCAACGCGTT TAGCAGGTCG GGCGCCGATA TCTTGAAGTC GCAGTTGGGG
       GGTTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC

21301  CCTCCGCCCT GCGCGCGCGA GTTGCGATAC ACAGGGTTGC AGCACTGGAA
       GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351  CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTCGGAGA
       GTGATAGTCG CGGCCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401  TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC
       AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451  TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCAGGCT TTGAGTTGCA
       AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT

21501  CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCGGTC TGGGCGTTAG
       GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551  GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC
       CTATGTCGCG GACGTATTTT CGGAACTAGA CGAATTTTCG GTGGACTCGG

21601  TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT
       AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651  GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA
       CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701  TCTGCACCAC ATTTCGGCCC CACCGGTTCT TCACGATCTT GGCCTTGCTA
       AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT
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Figure 27W

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21751  GACTGCTCCT T[illegible]CGCGCG CTGCCCGTTT TCGCTCGTCA CATCCA[illegible]C
       CTGACGAGGA AGTCGCGCGC GACGGGCAAA AGCGAGCAGT GTAGGTAAAG

21801  AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT
       TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA

21851  CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC
       GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTGCGCGT CGGGCACCCG

21901  TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG
       AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TGCGGACGTC

21951  GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT
       CTTAGCGGGG TAGTAGCAGT GTTTCCAGAA CAACGACCAC TTCCAGTCGA

22001  GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCATAC GGCCGCCAGA
       CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT

22051  GCTTCCACTT GGTCAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC
       CGAAGGTGAA CCAGTCCGTC ATCAAACTTC AAGCGGAAAT CTAGCAATAG

22101  CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC
       GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGGAAGAGGG

22151  ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT
       TGCGTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA

22201  TCCGCTTCGC TGGGCTCTTC CTCTTCCTCT TGCGTCCGCA TACCACGCGC
       AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCGCG

22251  CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC
       GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG

22301  CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCCACCAT TTGTAGCGCC
       GTACGAACTA ATCGTGGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG

22351  ACATCTTCTC TTTCTTCCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG
       TGTAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401  GCGCTCGGGC TTGGGAGAAG GGCGCTTCTT TTTCTTCTTG GGCGCAATGG
       CGCGAGCCCG AACCCTCTTC CCGCGAAGAA AAAGAAGAAC CCGCGTTACC

22451  CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC
       GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG

22501  AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT
       TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA

22551  CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG
       GTAGGCGAAA AAACCCCCGC GGGCCCCTCC GCCGCCGCTG CCCCTGCCCC

22601  ACGACACGTC CTCCATGGTT GGGGGACGTC GCGCCGCACC GCGTCCGCGC
       TGCTGTGCAG GAGGTACCAA CCCCCTGCAG CGCGGCGTGG CGCAGGCGCG

22651  TCGGGGGTGG TTTCGCGCTG CTCCTCTTCC CGACTGGCCA TTTCCTTCTC
       AGCCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG
```

Figure 27X

22701 CTATAGGCAG A[redacted]AGATCA TGGAGTCAGT CGAGAAGAAG GACAGC[redacted]A
GATATCCGTC TTTTTCTAGT ACCTCAGTCA GCTCTTCTTC CTGTCGGATT

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCCG CTTGAGGAGG AGGAAGTGAT
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG
ATAGCTCGTC CTGGGTCCAA AACATTCGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG
ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTGCGTCT CCGTTTGCTC

22951 GAACAAGTCG GGCGGGGGGA CGAAAGGCAT GGCGACTACC TAGATGTGGG
CTTGTTCAGC CCGCCCCCCT GCTTTCCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG
TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTCACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT
TCTTTTGCCG TGTACGCTCG GGTTGGGCGC GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAAACTGC
AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTTTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT
TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTCGTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG
CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTTGGT
CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCACCCA CTTTGCCTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

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23651  ACGAGCAGCT A[redacted]CGCTGG CTTCAAACGC GCGAGCCTGC CGACTT[redacted]G
       TGCTCGTCGA TCGCGCGACC GAAGTTTGCG CGCTCGGACG GCTGAACCTC

23701  GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA
       CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751  GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG
       CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801  AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG
       TTTGTAACGT GATGTGGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTC

23851  ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA
       TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901  CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG
       GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951  CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC
       GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAAGA TACGATGTGG

24001  TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT
       ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA

24051  CAAGGAGCTG CAGAAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG
       GTTCCTCGAC GTCTTTGACG ATTTCGTTTT GAACTTCCTG GATACCTGCC

24101  CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC
       GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAAGGGG

24151  GAACGCCTGC TTAAAACCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA
       CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201  AAGCATGTTG CAGAACTTTA GGAACTTTAT CCTAGAGCGC TCAGGAATCT
       TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251  TGCCCGCCAC CTGCTGTGCA CTTCCTAGCG ACTTTGTGCC CATTAAGTAC
       ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301  CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC
       GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351  CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG
       GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401  GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC
       CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451  CTGGTTTGCA ATTCGCAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT
       GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501  TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA
       ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551  AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT
       TTGAGTGAGG CCCCGACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA
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Figure 27Z

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24601  GAGGACTACC ACGCCCACGA GATTAGGTTC TACGAAGACC AATCCCGCCC
       CTCCTGATGG TGCGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651  GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG
       CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC

24701  GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG
       CGGTTAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC

24751  GGACGGGGGG TTTACTTGGA CCCCCAGTCC GGCGAGGAGC TCAACCCAAT
       CCTGCCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA

24801  CCCCCCGCCG CCGCAGCCCT ATCAGCAGCA GCCGCGGGCC CTTGCTTCCC
       GGGGGGCGGC GGCGTCGGGA TAGTCGTCGT CGGCGCCCGG GAACGAAGGG

24851  AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA
       TCCTACCGTG GGTTTTTCTT CGACGTCGAC GGCGGCGGTG GGTGCCTGCT

24901  GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGGAC GAGGAGGAGG
       CCTCCTTATG ACCCTGTCAG TCCGTCTCCT CCAAAACCTG CTCCTCCTCC

24951  AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC
       TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001  GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTCGCAT TCCCCTCGCC
       CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051  GGCGCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC
       CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101  CTCAGGCGCC GCCGGCACTG CCCGTTCGCC GACCCAACCG TAGATGGGAC
       GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG

25151  ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA
       TGGTGACCTT GGTCCCGGCC ATTCAGGTTC GTCGGCGGCG GCAATCGGGT

25201  AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG
       TCTCGTTGTT GTCGCGGTTC CGATGGCGAG TACCGCGCCC GTGTTCTTGC

25251  CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTCGCCCGC
       GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301  CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCCGTA ACATCCTGCA
       GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT

25351  TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA
       AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCCG TCGCCGTCGT

25401  ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC
       TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG

25451  AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC
       TTTCGGGTTC TTTAGGTGTC GCCGCCGTCG TCGTCCTCCT CCTCGCGACG

25501  GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT
       CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCCTAA
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Figure 27 AA

25551 TTTCCCACTC T[redacted]TGCTAT ATTTCAACAG AGCAGGGGCC AAGAACA[redacted]A
AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT

25601 GCTGAAAATA AAAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT
CGACTTTTAT TTTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTCGGCGCA CGCTGGAAGA CGCGGAGGCT
TAGTGTTTTC GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT
GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCCTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG
AAGAGTTTAA ATTCGCGCTT TTGATGCAGT AGAGGTCGCC GGTGTGGGCC

25801 CGCCAGCACC TGTTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC
GCGGTCGTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGCGGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCA
ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT
TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCGAA ACCGAATTCT CCTGGAACAG
GGGCCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC
CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC
GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAACTCAGG GGCGCAGCTT
GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTCACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA
CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTCAGCT CAACGACGAG TCGGTGAGCT
GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC
GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAACTCTGC AGACCTCGTC
GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT
GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCCTTCT CGGGACCTCC CGGCCACTAT
AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG
GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTTCCTGA GCCGCCTGCC

Figure 27 AB

26501 CTACGACTGA A[redacted]TAAGTG GAGAGGCAGA GCAACTGCGC CTGAAA[redacted]C
GATGCTGACT TACAATTCAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGGCCCGG CGCACGGCGT
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCTCACT
GGGTCGCGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAAATATA CTGGGGCTCC
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGGTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCGTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT
ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCTG CTGTGTGCAC ATTTGCATTT
TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA
TAACAGTCGA AAAATTTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTC

Figure 27AC

27451 GTGGATTTTA A[redacted]GCCAGC CTGTAATGTT ACATTCGCAG CTGAAG[redacted]A
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA
ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCCTGCTC AATACCATTC
TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAACT

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA
GCCTAAACAA GGTCAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA
CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28401 CATGTTCTTT T[redacted]TTACAG TATGATTAAA TGAGACATGA TTCCTC[redacted]T
GTACAAGAAA AGAGAATGTC ATACTAATTT ACTCTGTACT AAGGAGCTCA

28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG
AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT
GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA

28551 TTGCTTTACG GATTTGTCAC CCTCACGCTC ATCTGCAGCC TCATCACTGT
AACGAAATGC CTAAACAGTG GGAGTGCGAG TAGACGTCGG AGTAGTGACA

28601 GGTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT
CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT
TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA

28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTTCTGCT GATTATTTGC
TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC
TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAAA
TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT

28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC
CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG
ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC

28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC
CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT
AAGGTGACGT TGTTCAACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051 CGCCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG
GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG
TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC

29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA
GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201 TCAAGAGCTC CAAGACATGG TTAACTTGCA CCAGTGCAAA AGGGGTATCT
AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCCATAGA

29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA
AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCATT ATGGTGGCCT

29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT
GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27 AE

29351 GGTGGGAGAA A[redacted]CCATTA CCATAACTCA GCACTCGGTA GAAACC[redacted]G
CCACCCTCTT TTCGGGTAAT GGTATTGAGT CGTGAGCCAT CTTTGGCTTC

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT
CGACGTAAGT GAGTGGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT
TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACTTTC TCCACAATCT AAATGGAATG TCAGTTTCCT
GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC
GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACTG TGCCTTTTCT TACTCCTCCC TTTGTATCCC
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCCTGGGG TACTCTCTTT GCGCCTATCC
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG
CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAAT GTAACCACTG
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GGCGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGCACGA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG
GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTCAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA
AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA
CATCGAACCC GTAACTGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30301 CTAGGACTAA A[illegible]CGGGGC TCCTTTGCAT GTAACAGACG ACCTAA[illegible]C
GATCCTGATT T[illegible]GCCCCG AGGAAACGTA CATTGTCTGC TGGATT[illegible]TG

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACTAAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA
TTTGATTTCA ATGACCTCGG AACCCAAAAC TAAGTGTTCC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT
GAATTACATC GTCCTCCTGA TTCCTAACTA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC
TGAACTACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAAACTCAG CCCACAACTT GGATATTAAC
ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CCAAAAAGCT
ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTTGTTAA GGTTTTTCGA

30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA
ACTCCAATTG GATTCGTGAC GGTTCCCCAA CTACAAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA
ATCGGTAATT ACGTCCTCTA CCCGAACTTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTC
TTGTGTTTAG GGGAGTTTTG TTTTTAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA
TTTGTTCCGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACTGTCGT

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG
GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTCGA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG

30951 TAAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG
ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTCAGTTTT GGCTGTTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT
AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA
GTTTCACGAG TAGAATAATA TTCTAAACTG CTTTTACCTC ACGATGATTT

31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAAACTGCC AAAAGTAACA TTGTCAGTCA
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AA[illegible]GAGACA AAACTAAACC TGTAACACTA ACCATTA[illegible]C
TCAAATGAAT TTGCCTCTGT TTTGATTTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG
ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTCACG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC
AGTAAAAGTA CCCTGACCAG ACCGGTGTTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT
GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTTCTTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA
CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC
AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCCTAG TATTCAACCT GCCACCTCCC
GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCGGCTGG CCTTAAAAAG
AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG
GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCCTGTCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC
AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG
TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA
AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC
ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTCG

31901 AGCGCGCGAA TAAACTGCTG CCGCCGCCGC TCCGTCCTGC AGGAATACAA
TCGCGCGCTT ATTTGACGAC GGCGGCGGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC
GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCCT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA
CGGAACAGGA GGCCCGTGTC GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA
GTCATTGACG TCGTGTCGTG GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GGCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT
CCGCGACATA GGTTTCGAGT ACCGCCCCTG GTGTCTTGGG TGCACCGGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG
GTATGGTGTT CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32201 GACATAAACA T[illegible]CTCTTT TGGCATGTTG TAATTCACCA CCTCCC[illegible]A
CTGTATTTGT AATGGAGAAA ACCGTACAAC ATTAAGTGGT GGAGGGCCAT

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC
GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG

32301 AGCTGGCCAA AACCTGCCCG CCGGCTATAC ACTGCAGGGA ACCGGGACTG
TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT
CTTGTTACTG TCACCTCTCG GGTCCTGAGC ATTGGTACCT AGTAGTACGA

32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC
GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG

32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGGAACAACC
AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTTGG

32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA
GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT
TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

32601 CCTCCAGTAT GGTAGCGCGG GTTTCTGTCT CAAAAGGAGG TAGACGATCC
GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCCTCC ATCTGCTAGG

32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTTG GTCGTAGTGT
GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAC CAGCATCACA

32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCCTGAA GCAAAACCAG
GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG
CACGCCCGCA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC
GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

32851 CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA
GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCGTT
TGTAGGTGGT GGCGTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA

32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT
GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA

33001 TTTTTTTATT CCAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA
AAAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT

33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAACTCTACA GCCAAAGAAC
CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG

33101 AGATAATGGC ATTTGTAAGA TGTTGCACAA TGGCTTCCAA AAGGCAAACG
TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

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33151  GCCCTCACGT ([redacted]GTGGAC GTAAAGGCTA AACCCTTCAG GGTGAA[redacted]C
       CGGGAGTGCA G[redacted]CACCTG CATTTCCGAT TTGGGAAGTC CCACTT[redacted]AG

33201  CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC
       GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251  GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC
       CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG

33301  ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG
       TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGAAGTCGG AGTTCGTCGC

33351  AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA
       TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401  AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG
       TTCGCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451  CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC
       GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501  CCGCCAGGAA CCATGACAAA AGAACCCACA CTGATTATGA CACGCATACT
       GGCGGTCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551  CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG
       GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCGAACA ACGTACCCGC

33601  GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC
       CGCTATATTT TACGTTCCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG

33651  AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG
       TTTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701  CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG
       GAGGCCTTGG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751  CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT
       GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA

33801  TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG
       ATCTTCGGAC AGAATGTTGT CCTTTTTGTT GGGAATATTC GTATTCTGCC

33851  ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA
       TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT

33901  AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT
       TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951  CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG
       GCCATTTGTG TAGTCCAACT AAGTGTAGCC AGTCACGATT TTTCGCTGGC

34001  AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC
       TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAATGTCG

34051  CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC
       GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG
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Figure 27AJ

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34101  CTGAAAAACC C[illegible]TGCCTA GGCAAAATAG CACCCTCCCG CTCCAG[illegible]A
       GACTTTTTGG G[illegible]ACGGAT CCGTTTTATC GTGGGAGGGC GAGGTC[illegible]T

34151  ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA
       TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT

34201  AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC
       TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251  AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA
       TCAGTGTCAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT

34301  AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
       TTTTTACTGC ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG

34351  GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCCTCAAA
       CGCTTGGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401  TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAACT
       AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA

34451  ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
       TGTTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGGA TGCAGTGGGC

34501  CCCCGTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC
       GGGGCAAGGG TGCGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

                                                 PacI
                                               ~~~~~~~~
34551  ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
       TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACTACTA CAATTAATTC

34601  AATTCGGATC TGCGACGCGA GGCTGGATGG CCTTCCCCAT TATGATTCTT
       TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651  CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG
       GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701  GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
       CGTCCATCTA CTGCTGGTAG TCCCTGTCGA AGTTCCGGTC GTTTTCCGGT

34751  GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
       CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG

34801  CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
       GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851  GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
       CTGTCCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG

34901  GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
       CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG

34951  CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
       GGAAGCCCTT CGCACCGCGA AAGAGTATCG AGTGCGACAT CCATAGAGTC

35001  TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG
       AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC
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Figure 27 AK

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35051  TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC
       AAGTCGGGCT GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101  CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT
       GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151  TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
       ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201  CTAACTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG
       GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251  AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
       TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301  AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC
       TTGGTGGCGA CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG

35351  GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT
       CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401  GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT
       CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451  ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA
       TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501  TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
       ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551  TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
       AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601  GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
       CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTCACGACG

35651  AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA
       TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701  ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
       TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751  GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
       CGGAGGTAGG TCAGATAATT AACAACGGCC CTTCGATCTC ATTCATCAAG

35801  GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
       CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851  TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
       ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901  TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
       AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951  CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
       GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG
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Figure 27AL

36001 TCATGGTTAT [redacted]AGCACTG CATAATTCTC TTACTGTCAT GCCATC[redacted]A
AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA CGGGATAATA
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTTCCCT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA
TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA
ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

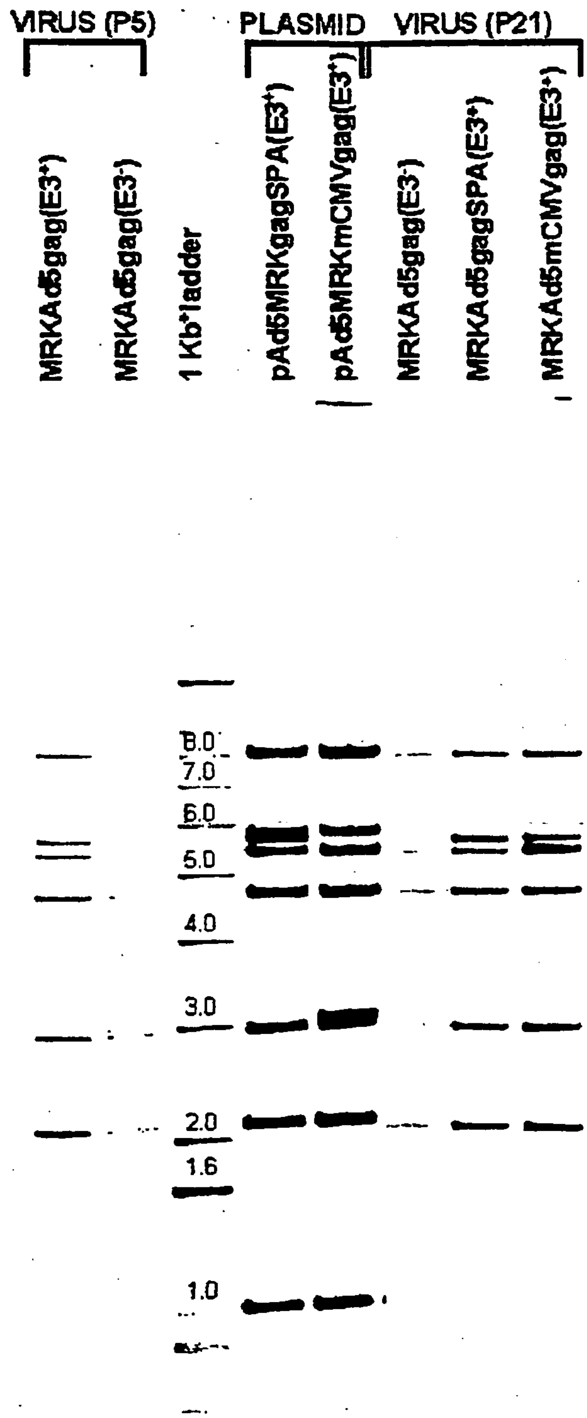
VIRUS (P5)
PLASMID
VIRUS (P21)
MRKAd5gag(E3+)
MRKAd5gag(E3-)
1 Kb+ladder
pAd5MRKgagSPA(E3+)
pAd5MRKmCMVgag(E3+)
MRKAd5gag(E3-)
MRKAd5gagSPA(E3+)
MRKAd5mCMVgag(E3+)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.6
1.0

FIGURE 28

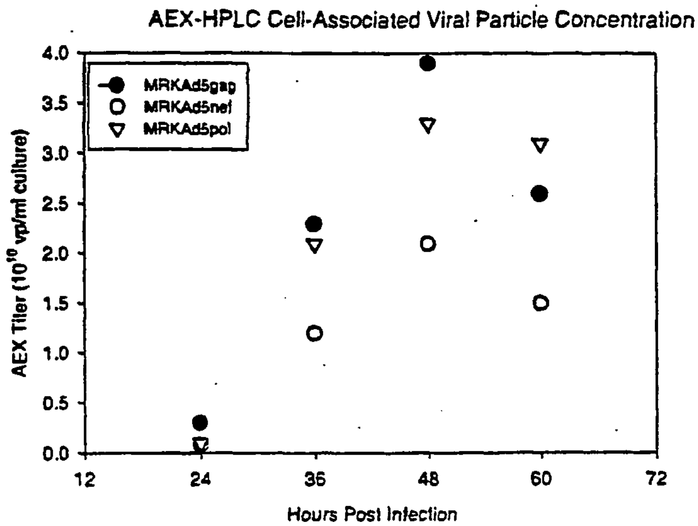

FIGURE 29A

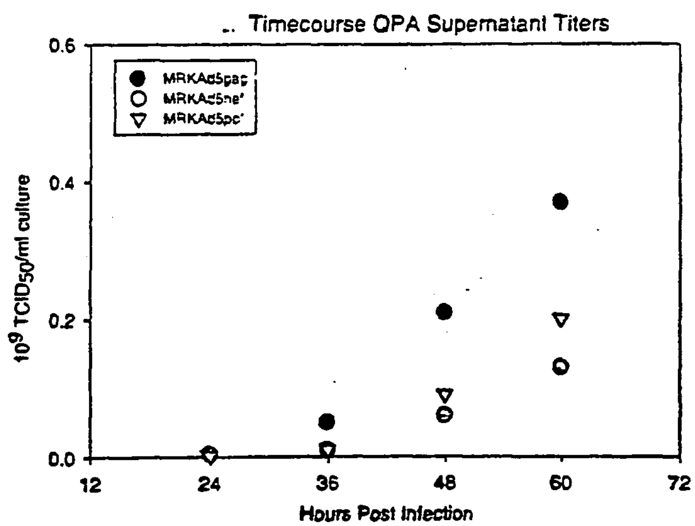

FIGURE 29B

atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga 48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

gca gtc ttc gtt tcg ccc agc gag atc tcc att gtg tgg gcc tcc agg 96
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg
20 25 30

gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag 144
Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu
35 40 45

ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc 192
Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly
50 55 60

tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt 240
Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys
65 70 75 80

gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag 288
Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys
85 90 95

att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct 336
Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala
100 105 110

gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg 384
Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val
115 120 125

cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc 432
Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr
130 135 140

ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag 480
Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu
145 150 155 160

gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac 528
Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp
165 170 175

ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag 576
Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln
180 185 190

atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg 624
Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu
195 200 205

cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc 672
His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro
210 215 220

agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att 720
Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile
225 230 235 240

ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag 768
Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys
245 250 255

Figure 30'A'

```
agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc      816
Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro
        260                 265                 270

acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac      864
Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp
        275                 280                 285

tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag      912
Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln
    290                 295                 300

gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac      960
Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn
305                 310                 315                 320

cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg     1008
Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu
                325                 330                 335

gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag     1056
Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys
            340                 345                 350

gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc     1104
Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr
        355                 360                 365

atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag     1152
Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys
    370                 375                 380

tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc     1200
Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala
385                 390                 395                 400

ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg     1248
Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met
                405                 410                 415

aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc     1296
Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro
            420                 425                 430

tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc     1344
Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro
        435                 440                 445

aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc     1392
Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr
    450                 455                 460

ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc     1440
Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala
465                 470                 475                 480

tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482
Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln  *  (SID NO:37)
                485                 490
```

Figure 30 B

Figure 31

IFN-γ Secretion against Gag 20-aa pool from CD3+ T cells of Monkey PBMCs

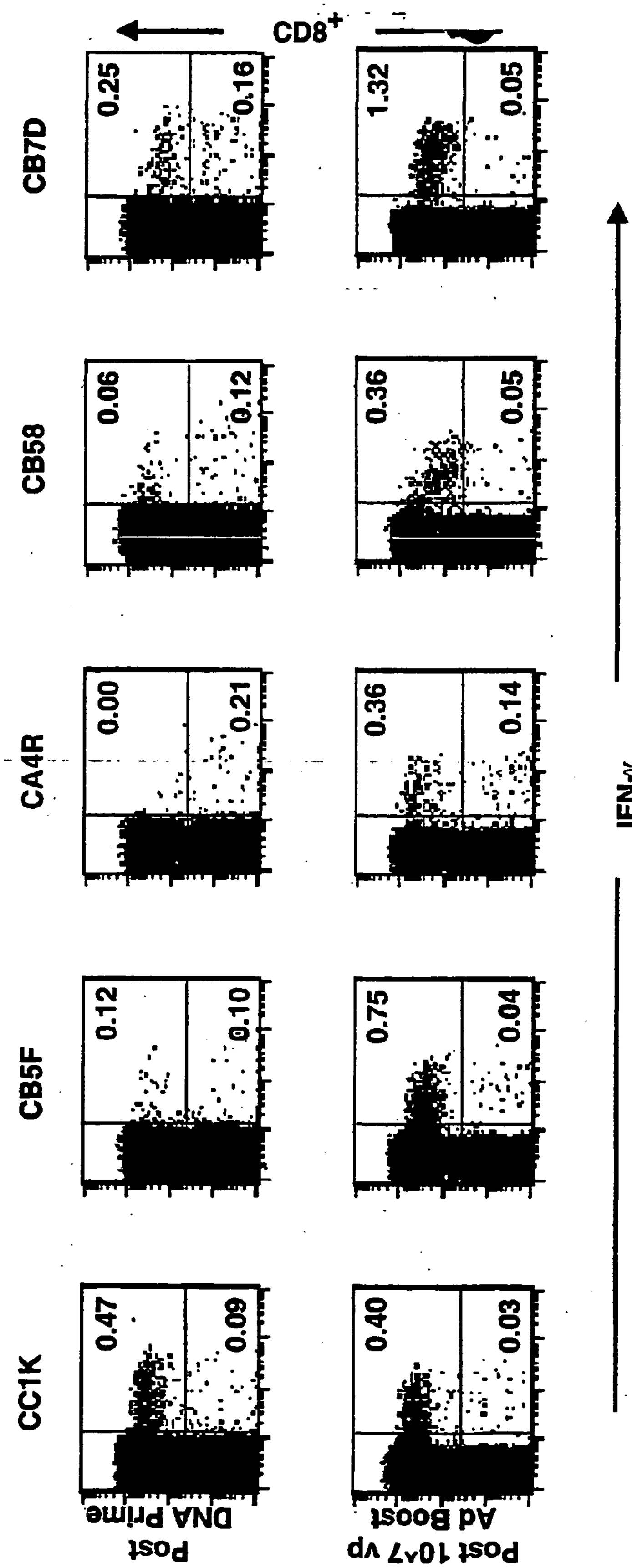

FIGURE 32

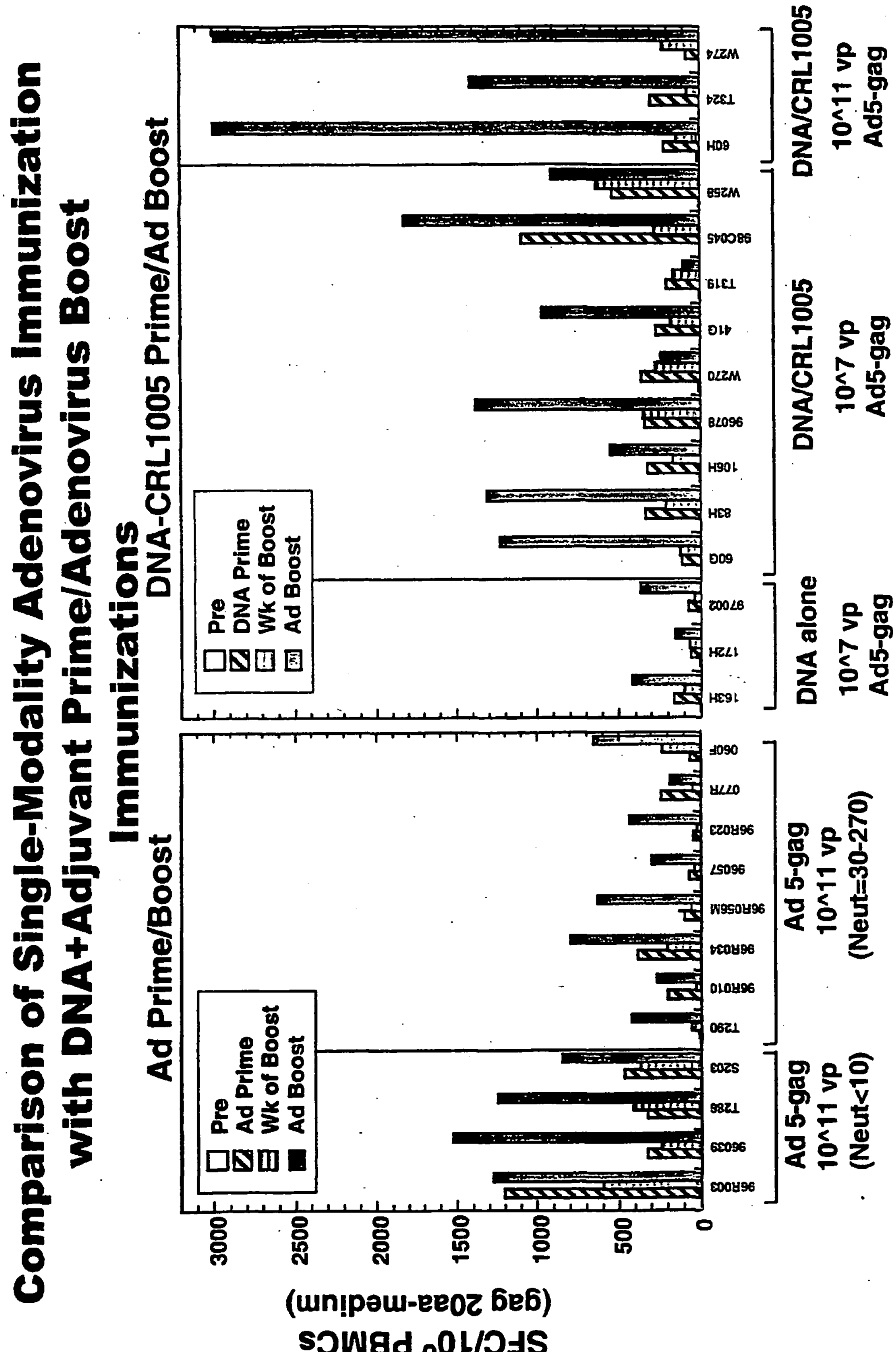
Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost Immunizations
Ad Prime/Boost
DNA-CRL1005 Prime/Ad Boost
Pre
Ad Prime
Wk of Boost
Ad Boost
DNA Prime
SFC/10^6 PBMCs (gag 20aa-medium)
0
500
1000
1500
2000
2500
3000
96R003
96039
T288
S203
T290
96R010
96R034
96R056M
96057
96R023
077R
060F
163H
172H
97002
60G
83H
106H
96078
W270
41G
T319
98C045
W258
60H
T324
W274
Ad 5-gag 10^11 vp (Neut<10)
Ad 5-gag 10^11 vp (Neut=30-270)
DNA alone 10^7 vp Ad5-gag
DNA/CRL1005 10^7 vp Ad5-gag
DNA/CRL1005 10^11 vp Ad5-gag

FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG
CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG
CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC
CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC
ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC
CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCCAGCA GGCTGCTGCT
GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC
CAGATGGTGC ACCAGGCCAT CTCCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG
GAGAAGGCCT TCTCCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC
CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG
CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT
GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC
TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCCATCCC TGTGGGGGAA
ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC
TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTC
TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC
CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT
GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC
AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG
GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC
ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC
CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC
CACAAGGGCA GGCCTGGCAA CTTCCTCCAG TCCAGGCCTG AGCCCACAGC CCCTCCCGAG
GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC
AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCCAG
ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC
CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC
ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC
CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT
GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG
CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG
ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC
ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC
AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC
AAGAAGCACC AGAAGGAGCC CCCCTTCCTG TGGATGGGCT ATGAGCTGCA CCCCGACAAG
TGGACTGTGC AGCCCATTGT GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG
AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG
GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG
GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG
GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GCCAGGTGGA CTGCTCCCCT
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG
GCTGTGTTCA TCCACAACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACTC TGACATCAAG
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys
Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp
Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser
Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln
Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln
Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser
Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys
Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His
Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser
Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn
Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly
Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala
Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala
Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met
Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly
Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp
Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn
Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln
Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu
Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu
Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile
Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys
Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys
Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr
Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu
Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr
Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr
Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met
Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln
Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr
Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp
Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp
SEQ ID NO: 39

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/22080 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/28861

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 15/86
US CL : 435/456
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6,10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 ---------- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 ---------- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X,P	US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 ---------- 4,5,13-17, 29, 30, 32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 06 February 2002 (06.02.2002)	Date of mailing of the international search report 13 MAR 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Ulrike Winkler, Ph.D. Telephone No. 703-308-0196

International application No.
PCT/US01/28861

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 31
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
This claim could not be searched because applicant did not provide a CRF.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.0, STN-BIOSIS, MEDLINE
adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

		and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type

		and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *nef-pol* fusion and one expressing *gag*.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *gag-pol* fusion and one expressing *nef*.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *nef-gag* fusion and one expressing *pol*.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant (for all designated States except US): **MERCK & CO., INC.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

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(75) Inventors/Applicants (for US only): **EMINI, Emilio, A.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **YOUIL, Rima** [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **BETT, Andrew, J.** [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CHEN, Ling** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **KASLOW, David, C.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **SHIVER, John, W.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **TONER, Timothy, J.** [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). **CASIMIRO, Daniel, R.** [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: **MERCK & CO., INC.**; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 --- 4, 5, 13-17, 29-32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 --- 4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 --- 4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 06 February 2002 (06.02.2002)	Date of mailing of the international search report 19 AUG 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Ulrike Winkler, Ph.D. Della Collins for Telephone No. 703-308-0196

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immmodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1

		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *nef-pol* fusion and one expressing *gag*.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *gag-pol* fusion and one expressing *nef*.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from two individual vectors, one expressing *nef-gag* fusion and one expressing *pol*.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein *gag, pol* and *nef* are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.0, STN-BIOSIS, MEDLINE
adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/022080 A3

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replication-defective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. $CD8^+$ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive $CD8^+$ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from $CD4^+$ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag, pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH_2-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication –defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a);and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to – highly active antiretroviral therapy –.

"first generation" vectors are characterized as being replication-defective. They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BglII* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BglII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH_2-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of $CD3^+$ cells that were $CD8^+\gamma IFN^+$ and $CD4^+\gamma IFN^+$, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator..

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic seqeunces, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 μg and 200 μg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH_2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6® cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6®. Both these cell lines express the adenoviral E1 gene product. PER.C6® is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6®, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM $MgCl_2$; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM $MgCl_2$, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *Msc*1 site of the hCMV promoter and a 3' primer (designed to contain the B*gl*II recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *Msc*1 and B*gl*II. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *Msc*1 and *Bgl*II digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *Bgl*II digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *Bgl*II site. Colonies were screened using *Sma*1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, $(GT)_n$, and $(T)_n$; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901[a]	10.8
PVIJns-hCMV-FLgag-bGHpA[b]	16.6
pV1Jns-hCMV-FLgag-SPA[b,c]	12.0

[a] GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

[b] New plasmid constructions that have the intron A portion removed from the hCMV promoter.

[c] In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 μg and 200 μg.

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA[a] Promoter/terminator	Dose, ug[b]	Anti-p24 Titers (3 Wk PD1)[c]			SFC/10^6 Cells (4 Wk PD1)[d]		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV-FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV-FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971,	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

[a]in PBS
[b]i.m. Injections into both quads, 50 µL per quad
[c]n=10;GMT, geometric mean titer; SE, standard. error
[d]n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector -"MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

(1) The left ITR region was extended to include the *Pac*1 site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.

(2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.

(3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac*1 and *BstZ*1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*1 linearized pAdHVO (E3- adenovector) or *Cla*1 linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI , BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and subsequently labeled with [^{33}P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and then labeled with [^{33}P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *Msc*1 overnight and then digested with *Sfi*1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*1. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH_20. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was *Pac*1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6® cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [^{33}P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac*1/*Hind*III prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10^6 cells/ml), Viability (%) Infection	Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^8 $TCID_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 80%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.95, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.76, 69%	50	52	8.2	4.7	1.70	81	170	
P8	1.03, 94%	0.86, 84%	47.5	64	9.0	8.7	1.10	82	310	
P9	0.69, 95%	0.99, 73%	47.5	58	4.4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.06, 65%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	6.5	1.20	45	200	2.86 2.60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 92%	0.68, 67%	46	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10^6 cells/ml), Viability (%) Infection	Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^8 $TCID_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1.55, 86%	1.28, 76%	49.5	50	1.2	0.8	0.58	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.18	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 86%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.6	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.96, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	8.7			350	3.12 2.91
P15	0.87, 99%	0.97, 89%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

MRKAd5gag(E3-)

	Xv (10^6 cells/ml), Viability (%) Infection	Xv (10^6 cells/ml), Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10^{10} vp/ml culture	Titer 10^4 vp/cell	QPA 10^9 $TCID_{50}$/ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 96%	0.88, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 91%	0.57, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P16	0.87, 89%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors[a]	µg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag[b]	1.40
Clinical lot Ad5gag[c]	1.28
Research lot Ad5gag[d]	1.32
MCMVFL-gagbGHpA[e]	0.42

[a] A_{260nm} absorbance readings taken for viral particle determinations.
[b] MRKAd5gag was produced in serum free conditions and purified at P5.
[c] Clinical lot# Ad5gagFN0001
[d] Research Ad5FLgag lot# 6399
[e] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	[a]MRKAd5gag	10^7	25600	5877	4780
2	"	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	"	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	"	10^9	310419	99181	75165
7	[b]mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	"	10^9	941014	239068	190836
9	[c]hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10	"	10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12	"	10^7	14703	5274	3882
13	"	10^8	58813	14942	11915
14	"	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16	"	10^7	4222	3405	1138
17	"	10^8	19401	3939	3274
18	"	10^9	89144	25187	19639
19	Naïve	none	93	7	6

*2x50 µL i.m. (quad) injections/animal
P.I.s: Youil, Chen, Casimiro
Vaccination: T. Toner, Q. Su
Assay: M. Chen

[a]The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).
[b]The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) ws used here.
[c]This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^{9} vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag[a], 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag[b], Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
[a]MRKAd5gag (hCMV, bGHpA, E3+)								
[b]original Ad5gag vector (hCMV/intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of $CD4^+$ T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media[a]	Gag H[b]	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag	97N010	6	89	0	395	0	1058	0	1174	3	775	4	1074
	10^11 vp	97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	396	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2676			0	1656	0	1278
2	MRKAd5gag	97N120	5	275	1	249	4	141	4	119	9	206	4	219
	10^9 vp	97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot	97X001	0	281	1	485	0	817	0	1220b	1	894	0	1858
	10^11 vp	97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot	97N020	3	30	1	101	0	66	0	36	0	26	0	41
	10^9 vp	97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Naïve	96R041	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	19	15	10	15	24	9

Based on either 4x10^5 or 2x10^5 cells per well (depending on spot density)
ND. not determined
[a]mock or no peptide control
[b]Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGA GTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

wt aa	aa residue	mutant aa	enzyme function
Asp	112	Ala	RT
Asp	187	Ala	RT
Asp	188	Ala	RT
Asp	445	Ala	RNase H
Glu	480	Ala	RNase H
Asp	500	Ala	RNase H
Asp	626	Ala	IN
Asp	678	Ala	IN
Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID NO:3).

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.* 1989; 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
CCCTGAGTGG GAGTTTGTGA ACACCCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH_2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

```
CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID N0:11).
```

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

```
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).
```

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC
(SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BglII* site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *BglII* site. The clones were checked for the correct orientation of the gene by using restriction enzymes *DraIII/Not1*. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac1* and *Bst1107* I (or its isoschizomer, *BstZ107* I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla1* digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAd5pol was digested with restriction enzyme *PacI* (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ –60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac*1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ –60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA AT<u>G CGG CCG C</u>CA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II)Reverse: 5'-AAG GA<u>A GAT CT</u>A CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA AT<u>G GCG CGC C</u>AT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GA<u>A GAT CT</u>A CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc*1 and *Bgl*11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc*1/*Bgl*11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl*11 and the gag reporter gene (*Bgl*11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*1 and *Bst*Z1101 digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT <u>GGA TCC</u> ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA <u>GGA TCC</u> TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *BamHI*, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM $MgCl_2$, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μL of 1 μg /mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 µL of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 µL 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 µL of 0.5M H_2SO4 per well. OD_{492} readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD_{492} (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at $5x10^6$/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 µL/well of either 5 µg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 µL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 µL of cell samples ($4\text{-}5x10^5$ cells per well) and 50 µL of the antigen solution were added. To the control well, 50 µL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of $CD4^+$-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of $CD8^+$-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 ($CD8^+$ T cell epitope) or aa81-100 ($CD4^+$) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO_2, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10^6 cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN_3) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific $CD4^+$ and $CD8^+$ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers[a]			SFC/10^6 cells[c]		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2 1	1638400[b] 713155	0 528520	0 303556	2(2) 1(1)	114(9) 48(7)	2083(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2 1	310419 6400	388218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2 1	1638400[b] 1241675[b]	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

[a]GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean
[b]Near or at the upper limit of the serial dilution; hence, could be greater than this value
[c]No. of Spot-forming Cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers[a]			SFC/10^6 cells[b]		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10^9 vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10^7 vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	62	21(2)	18(6)	28(3)

[a]GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean
[b]No. of spot-forming cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IApol(E3+) 10^11 vp	99C100	1	0	0	1	38	31	0	52	146	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+) 10^9 vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	192	4	38	158	5	38	108
	99C201	8	5	21	8	62	62	0	18	32	1	14	65
MRKAd5hCMV-IApol(E3-) 10^11 vp	99D239	5	2	2	20	82	172	1	68	114	9	21	40
	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	236	1	24	284
MRKAd5hCMV-IApol(E3-) 10^9 vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Naive	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10^11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10^9 vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10^11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10^9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naive	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

EXAMPLE 26
Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10^{10} vp/ml culture)	AEX Titer (10^4 vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%) Infection	Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10^6 cells/ml), Viability (%) Infection	Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10^{10} vp/ml culture	Titer 10^4 vp/cell	Amplification Ratio	Triton Lysis Titer 10^{10} vp/ml culture
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10^6 cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10^{10} vp/ml culture	10^4 vp/cell	Ratio	10^{10} vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2×10^6 cells/ml. Cells were grown until they reached a cell concentration of approximately 1×10^6 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi ($1x10^{13}$ vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi ($1x10^{11}$ IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$-biased or $CD8^+$-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly $CD8^+$-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag

Number of SFC/million PBMCs

Grp#	Priming T=0, 4, 8 wks	Boost T=28 wks	Monk#	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1	DNA/5 mgs	MRKAd5gag(E3+)	CB5H	NA	NA	3	35	15	71	4	224	8	115	6	05	19	956	0	316
	PBS	10^7 vp	CC6X	0	0	0	15	0	46	0	68	0	75	0	35	3	1705	1	755
	(D101)		AW3G	5	11	0	36	3	51	3	46	2	89	8	65	10	989	0	395
2	DNA/5mgs +	MRKAd5gag(E3+)	CC1C	0	4	1	60	0	111	5	270	4	280	8	232	3	959	19	1345
	CRL1005/45mgs	10^7 vp	CC1K	4	0	1	101	0	254	0	791	5	452	0	321	0	1915	1	1099
			AW3P	9	8	1	10	4	71	4	164	8	104	5	85	11	838	6	241
			CB5F	NA	NA	0	31	0	288	0	530	19	374	9	251	8	1549	20	1734
			AK8B	9	12	4	38	1	119	0	439	0	425	0	316	4	1229	5	1354
3	DNA/5 mgs+	MRKAd5gag(E3+)	AW20	10	4	1	59	5	264	19	425	6	105	9	205	18	565	8	404
	CRL1005/7.5 mgs + 0.6 mM BAK	10^7 vp	CA4R	1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
			CB58	8	6	0	6	3	119	0	274	6	282	1	208	0	636	1	828
			CB5W	4	3	0	26	1	91	0	139	0	164	1	62	5	643	1	349
			CB7D	1	0	0	136	0	316	1	609	5	626	1	769	0	2278	4	1831
4	none	None	98D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique Bam*HI* site. This fragment was digested with BstE*II* and Bam*HI*. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the Pst*I*-BstE*II* gag digestion fragment, the BstE*II*/Bam*HI* digested PCR product and long Pst*I*/Bam*HI* V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the Bgl*II* fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10^10 vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10^8 vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10^10 vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10^8 vp	AW38	1	-	19	30	-
		CC8K	0	-	50	895	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10^10 vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10^8 vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10^10 vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10^8 vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10^10 vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10^8 vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

:

1. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and

b) a gene encoding an HIV protein or immunologically relevant modification thereof.

2. A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.

3. A vector in accordance with claim 1 further comprising nucleotides corresponding to between from about base pair 3511 to about 3524 to about base pair 5798 of a wildtype adenovirus genome.

4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.

5. A vector in accordance with claim 4 which is deleted of base pairs 451-3510.

6. A vector in accordance with claim 1 which is at least partially deleted in E3.

7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6® cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) SEQ ID NO: 29;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6® cell.

62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

a) gag, pol, and nef, expressed independently from three individual vectors;

b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;

d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;

e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;

f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;

g) gag and pol, expressed independently from two individual vectors;

h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

i) pol and nef, expressed independently from two individual vectors;

j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

k) nef and gag, expressed independently from two individual vectors;

l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to a single promoter; and the encoding nucleic acid sequences operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

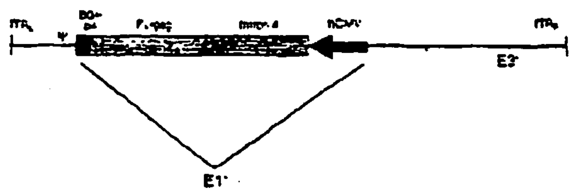

Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg
caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc
ctgctggagacctctgaggggtgcaggcagatcctgggccagctccagccctccctgcaaacaggctctgagg
agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc
acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag
gccatctcccccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc
catgttctctgccctgtctgagggtgccacccccccaggacctgaacaccatgctgaacacagtgggggggccatc
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acgctggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct
ccaggagcagattggctggatgaccaacaacccccccatccctgtgggggaaatctacaagaggtggatcat
cctgggcctgaacaagattgtgaggatgtactcccccacctccatcctggacatcaggcagggccccaaggag
cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact
ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg
ctgccaccctggaggagatgatgacagcctgccagggggtgggggggccctggtcacaaggccagggtgctg
gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag
gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga
agggctgctggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaggccaacttcctg
ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct
cccgaggagtccttcaggtttggggaggagaagaccacccccagccagaagcaggagcccattgacaagg
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gat (SEQ ID NO: 29)

Figure 2

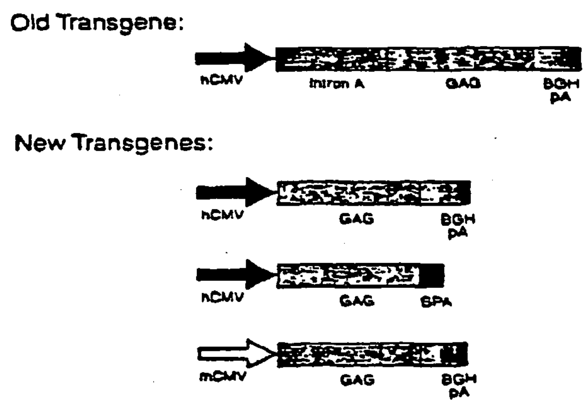


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

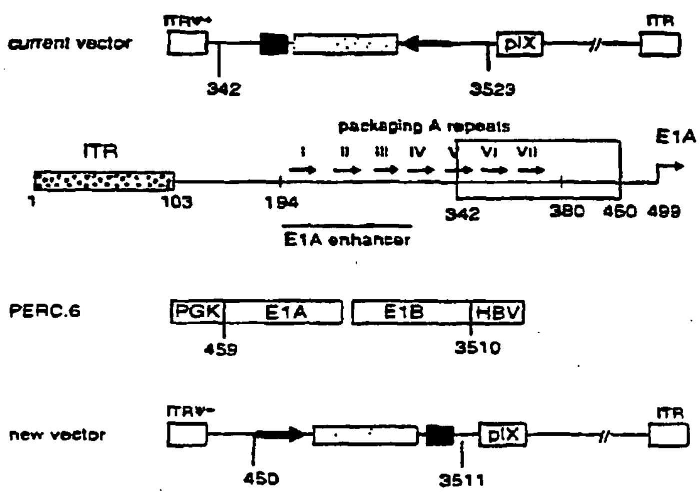


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

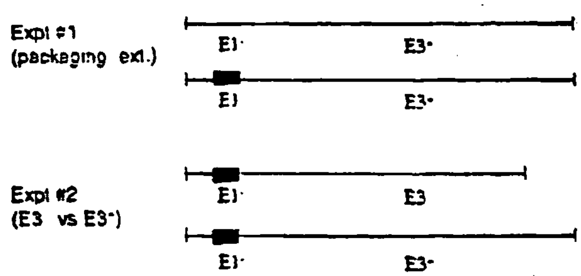


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

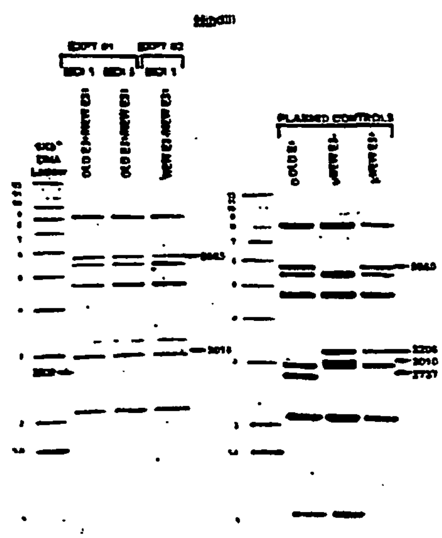


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

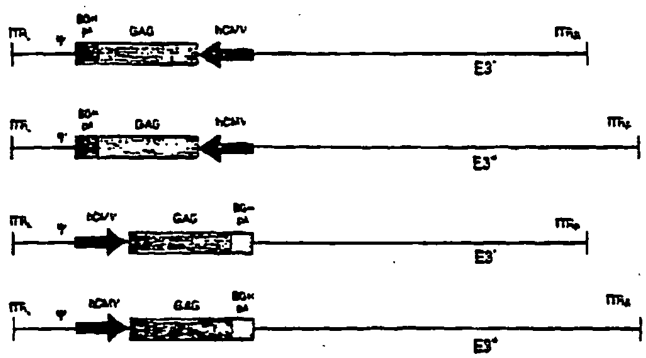


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

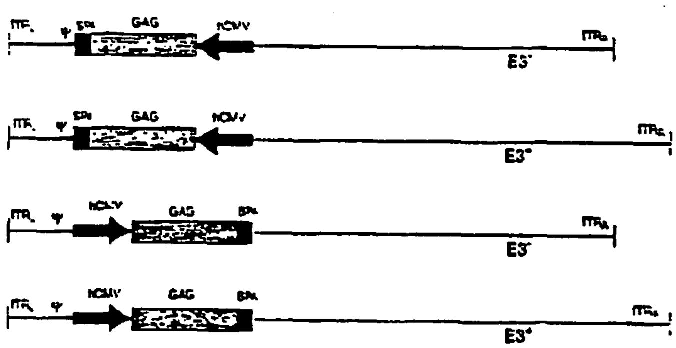


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

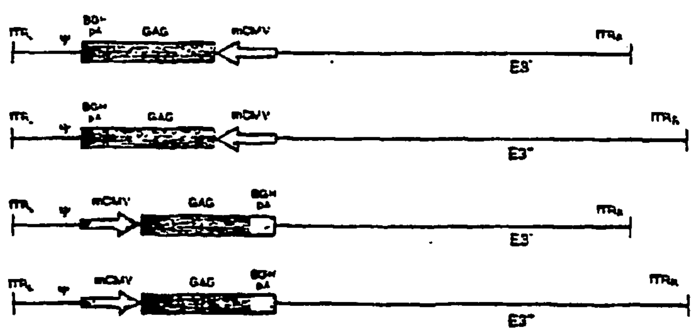


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

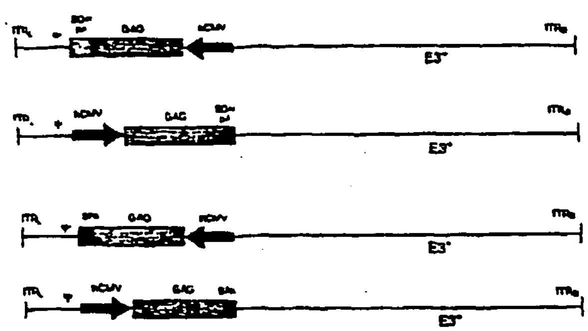


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

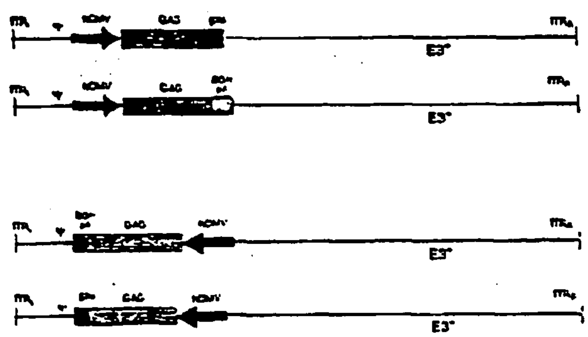


Figure 8B: Effect of polyadenylation signal

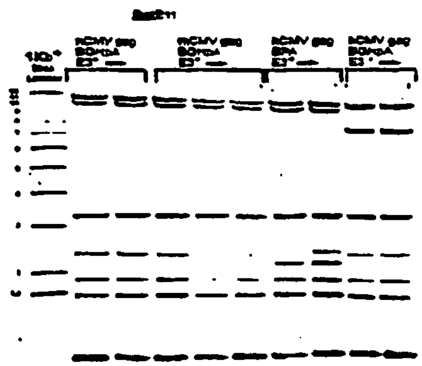


Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

<u>Hind</u> III

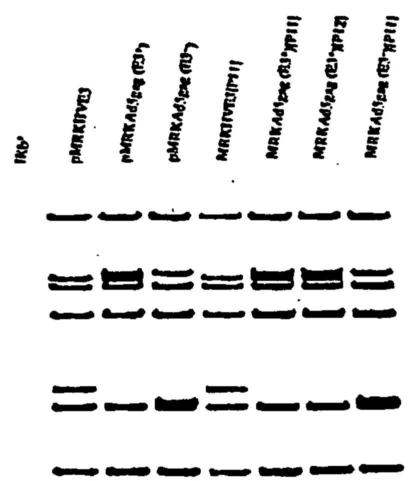


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

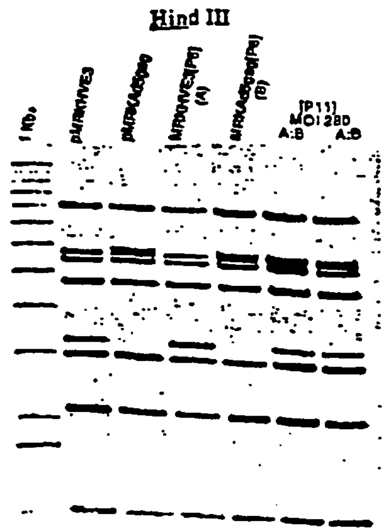


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

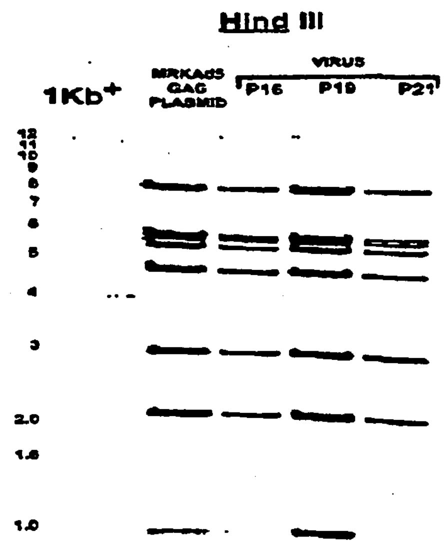
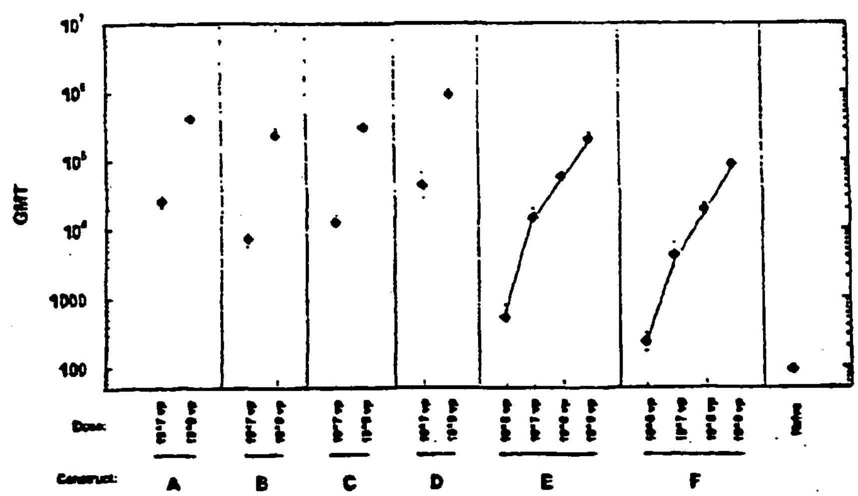


Figure 12: Viral DNA analysis by *Hin*dIII digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hin*dIII), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure 13. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁻ bCMV-FLgag-bGHpA; (C) MRKAd5 E3⁻ bCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



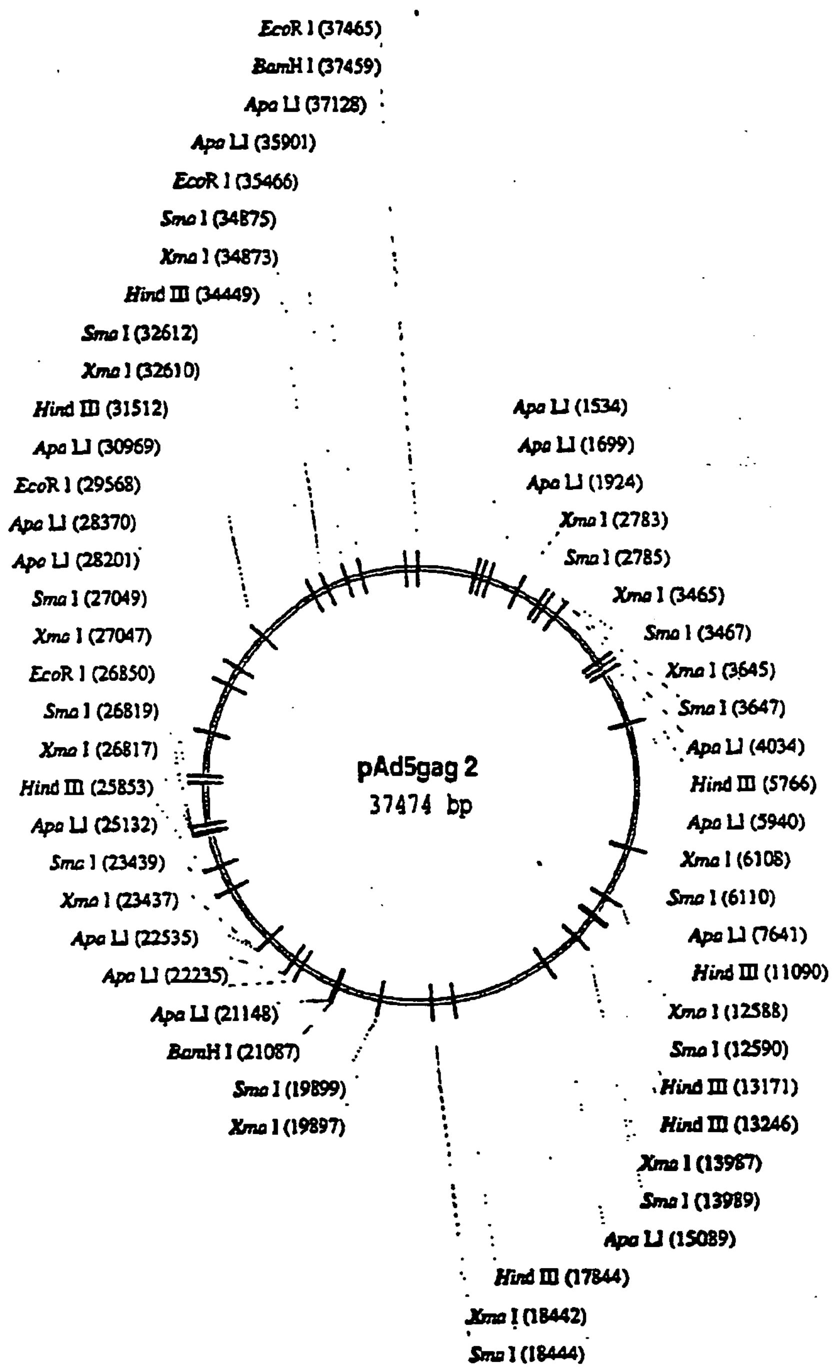

EcoR I (37465)
BamH I (37459)
Apa LI (37128)
Apa LI (35901)
EcoR I (35466)
Sma I (34875)
Xma I (34873)
Hind III (34449)
Sma I (32612)
Xma I (32610)
Hind III (31512)
Apa LI (30969)
EcoR I (29568)
Apa LI (28370)
Apa LI (28201)
Sma I (27049)
Xma I (27047)
EcoR I (26850)
Sma I (26819)
Xma I (26817)
Hind III (25853)
Apa LI (25132)
Sma I (23439)
Xma I (23437)
Apa LI (22535)
Apa LI (22235)
Apa LI (21148)
BamH I (21087)
Sma I (19899)
Xma I (19897)
Apa LI (1534)
Apa LI (1699)
Apa LI (1924)
Xma I (2783)
Sma I (2785)
Xma I (3465)
Sma I (3467)
Xma I (3645)
Sma I (3647)
Apa LI (4034)
Hind III (5766)
Apa LI (5940)
Xma I (6108)
Sma I (6110)
Apa LI (7641)
Hind III (11090)
Xma I (12588)
Sma I (12590)
Hind III (13171)
Hind III (13246)
Xma I (13987)
Sma I (13989)
Apa LI (15089)
Hind III (17844)
Xma I (18442)
Sma I (18444)
pAd5gag 2
37474 bp

Figure 14

pMRKAd5gag MER602

```
       PacI
1      TTCTTAATTA ACATCATCAA TAATATACCT TATTTTGGAT TGAAGCCAAT ATGATAATGA GGGGGTGGAG TTTGTGACGT GGCGCGGGGC GTGGGAACGG
       AAGAATTAAT TGTAGTAGTT ATTATATGGA ATAAAACCTA ACTTCGGTTA TACTATTACT CCCCCACCTC AAACACTGCA CCGCGCCCCG CACCCTTGCC
101    GGCGGGTGAC GTAGTAGTGT GGCGGAAGTG TGATGTTGCA AGTGTGGCGG AACACATGTA AGCGACGGAT GTGGCAAAAG TGACGTTTTT GGTGTGCGCC
       CCGCCCACTG CATCATCACA CCGCCTTCAC ACTACAACGT TCACACCGCC TTGTGTACAT TCGCTGCCTA CACCGTTTTC ACTGCAAAAA CCACACGCGG
201    GGTGTACACA GGAAGTGACA ATTTTCGCGC GGTTTTAGGC GGATGTTGTA GTAAATTTGG GCGTAACCGA GTAAGATTTG GCCATTTTCG CGGGAAAACT
       CCACATGTGT CCTTCACTGT TAAAAGCGCG CCAAAATCCG CCTACAACAT CATTTAAACC CGCATTGGCT CATTCTAAAC CGGTAAAAGC GCCCTTTTGA
301    GAATAAGAGG AAGTGAAATC TGAATAATTT TGTGTTACTC ATAGCGCGTA ATATTTGTCT AGGGCCGCGG GGACTTTGAC CGTTTACGTG GAGACTCGCC
       CTTATTCTCC TTCACTTTAG ACTTATTAAA ACACAATGAG TATCGCGCAT TATAAACAGA TCCCGGCGCC CCTGAAACTG GCAAATGCAC CTCTGAGCGG
401    CAGGTGTTTT TCTCAGGTGT TTTCCGCGTT CCGGGTCAAA GTTGGCGTTT TATTATTATA GGCGGCCGCG ATCCATTGCA TACGTTGTAT CCATATCATA
       GTCCACAAAA AGAGTCCACA AAAGGCGCAA GGCCCAGTTT CAACCGCAAA ATAATAATAT CCGCCGGCGC TAGGTAACGT ATGCAACATA GGTATAGTAT
501    ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA
       TATACATGTA AATATAACCG AGTACAGGTT GTAATGGCGG TACAACTGTA ACTAATAACT GATCAATAAT TATCATTAGT TAATGCCCCA GTAATCAAGT
601    TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT
       ATCGGGTATA TACCTCAAGG CGCAATGTAT TGAATGCCAT TTACCGGGCG GACCGACTGG CGGGTTGCTG GGGGCGGGTA ACTGCAGTTA TTACTGCATA
701    GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC
       CAAGGGTATC ATTGCGGTTA TCCCTGAAAG GTAACTGCAG TTACCCACCT CATAAATGCC ATTTGACGGG TGAACCGTCA TGTAGTTCAC ATAGTATACG
801    CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA
       GTTCATGCGG GGGATAACTG CAGTTACTGC CATTTACCGG GCGGACCGTA ATACGGGTCA TGTACTGGAA TACCCTGAAA GGATGAACCG TCATGTAGAT
901    CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC
       GCATAATCAG TAGCGATAAT GGTACCACTA CGCCAAAACC GTCATGTAGT TACCCGCACC TATCGCCAAA CTGAGTGCCC CTAAAGGTTC AGAGGTGGGG
1001   ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG
       TAACTGCAGT TACCCTCAAA CAAAACCGTG GTTTTAGTTG CCCTGAAAGG TTTTACAGCA TTGTTGAGGC GGGGTAACTG CGTTTACCCG CCATCCGCAC
1101   TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA
       ATGCCACCCT CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG CGGACCTCTG CGGTAGGTGC GACAAAACTG GAGGTATCTT CTGTGGCCCT
                                                                        BglII
1201   CCGATCCAGC CTCCGCGGCC GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGAGATCTAC CATGGGTGCT AGGGCTTCTG TGCTGTCTGG
       GGCTAGGTCG GAGGCGCCGG CCCTTGCCAC GTAACCTTGC GCCTAAGGGG CACGGTTCTC ACTCTAGATG GTACCCACGA TCCCGAAGAC ACGACAGACC
1301   TGGTGAGCTG GACAAGTGGG AGAAGATCAG GCTGAGGCCT GGTGGCAAGA AGAAGTACAA GCTAAAGCAC ATTGTGTGGG CCTCCAGGGA GCTGGAGAGG
       ACCACTCGAC CTGTTCACCC TCTTCTAGTC CGACTCCGGA CCACCGTTCT TCTTCATGTT CGATTTCGTG TAACACACCC GGAGGTCCCT CGACCTCTCC
1401   TTTGCTGTGA ACCCTGGCCT GCTGGAGACC TCTGAGGGGT GCAGGCAGAT CCTGGGCCAG CTCCAGCCCT CCCTGCAAAC AGGCTCTGAG GAGCTGAGGT
       AAACGACACT TGGGACCGGA CGACCTCTGG AGACTCCCCA CGTCCGTCTA GGACCCGGTC GAGGTCGGGA GGGACGTTTG TCCGAGACTC CTCGACTCCA
1501   CCCTGTACAA CACAGTGGCT ACCCTGTACT GTGTGCACCA GAAGATTGAT GTGAAGGACA CCAAGGAGGC CCTGGAGAAG ATTGAGGAGG AGCAGAACAA
       GGGACATGTT GTGTCACCGA TGGGACATGA CACACGTGGT CTTCTAACTA CACTTCCTGT GGTTCCTCCG GGACCTCTTC TAACTCCTCC TCGTCTTGTT
1601   GTCCAAGAAG AAGGCCCAGC AGGCTGCTGC TGGCACAGGC AACTCCAGCC AGGTGTCCCA GAACTACCCC ATTGTGCAGA ACCTCCAGGG CCAGATGGTG
       CAGGTTCTTC TTCCGGGTCG TCCGACGACG ACCGTGTCCG TTGAGGTCGG TCCACAGGGT CTTGATGGGG TAACACGTCT TGGAGGTCCC GGTCTACCAC
```

Figure 15A

pMRKAd5gag MER6B2

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1701  CACCAGGCCA TCTCCCCCCG GACCCTGAAT GCCTGGGTGA AGGTGGTGGA GGAGAAGGCC TTCTCCCCTG AGGTGATCCC CATGTTCTCT GCCCTGTCTG
      GTGGTCCGGT AGAGGGGGGC CTGGGACTTA CGGACCCACT TCCACCACCT CCTCTTCCGG AAGAGGGGAC TCCACTAGGG GTACAAGAGA CGGGACAGAC
1801  AGGGTGCCAC CCCCCAGGAC CTGAACACCA TGCTGAACAC AGTGGGGGGC CATCAGGCTG CCATGCAGAT GCTGAAGGAG ACCATCAATG AGGAGGCTGC
      TCCCACGGTG GGGGGTCCTG GACTTGTGGT ACGACTTGTG TCACCCCCCG GTAGTCCGAC GGTACGTCTA CGACTTCCTC TGGTAGTTAC TCCTCCGACG
1901  TGAGTGGGAC AGGCTGCATC CTGTGCACGC TGGCCCCATT GCCCCTGGCC AGATGAGGGA GCCCAGGGGC TCTGACATTG CTGGCACCAC CTCCACCCTG
      ACTCACCCTG TCCGACGTAG GACACGTGCG ACCGGGGTAA CGGGGACCGG TCTACTCCCT CGGGTCCCCG AGACTGTAAC GACCGTGGTG GAGGTGGGAC
2001  CAGGAGCAGA TTGGCTGGAT GACCAACAAC CCCCCCATCC CTGTGGGGGA AATCTACAAG AGGTGGATCA TCCTGGGCCT GAACAAGATT GTGAGGATGT
      GTCCTCGTCT AACCGACCTA CTGGTTGTTG GGGGGGTAGG GACACCCCCT TTAGATGTTC TCCACCTAGT AGGACCCGGA CTTGTTCTAA CACTCCTACA
2101  ACTCCCCCAC CTCCATCCTG GACATCAGGC AGGGCCCCAA GGAGCCCTTC AGGGACTATG TGGACAGGTT CTACAAGACC CTGAGGGCTG AGCAGGCCTC
      TGAGGGGGTG GAGGTAGGAC CTGTAGTCCG TCCCGGGGTT CCTCGGGAAG TCCCTGATAC ACCTGTCCAA GATGTTCTGG GACTCCCGAC TCGTCCGGAG
2201  CCAGGAGGTG AAGAACTGGA TGACAGAGAC CCTGCTGGTG CAGAATGCCA ACCCTGACTG CAAGACCATC CTGAAGGCCC TGGGCCCTGC TGCCACCCTG
      GGTCCTCCAC TTCTTGACCT ACTGTCTCTG GGACGACCAC GTCTTACGGT TGGGACTGAC GTTCTGGTAG GACTTCCGGG ACCCGGGACG ACGGTGGGAC
2301  GAGGAGATGA TGACAGCCTG CCAGGGGGTG GGGGGCCCTG GTCACAAGGC CAGGGTGCTG GCTGAGGCCA TGTCCCAGGT GACCAACTCC GCCACCATCA
      CTCCTCTACT ACTGTCGGAC GGTCCCCCAC CCCCCGGGAC CAGTGTTCCG GTCCCACGAC CGACTCCGGT ACAGGGTCCA CTGGTTGAGG CGGTGGTAGT
2401  TGATGCAGAG GGGCAACTTC AGGAACCAGA GGAAGACAGT GAAGTGCTTC AACTGTGGCA AGGTGGGCCA CATTGCCAAG AACTGTAGGG CCCCCAGGAA
      ACTACGTCTC CCCGTTGAAG TCCTTGGTCT CCTTCTGTCA CTTCACGAAG TTGACACCGT TCCACCCGGT GTAACGGTTC TTGACATCCC GGGGGTCCTT
2501  GAAGGGCTGC TGGAAGTGTG GCAAGGAGGG CCACCAGATG AAGGACTGCA ATGAGAGGCA GGCCAACTTC CTGGGCAAAA TCTGGCCCTC CCACAAGGGC
      CTTCCCGACG ACCTTCACAC CGTTCCTCCC GGTGGTCTAC TTCCTGACGT TACTCTCCGT CCGGTTGAAG GACCCGTTTT AGACCGGGAG GGTGTTCCCG
2601  AGGCCTGGCA ACTTCCTCCA GTCCAGGCCT GAGCCCACAG CCCCTCCCGA GGAGTCCTTC AGGTTTGGGG AGGAGAAGAC CACCCCCAGC CAGAAGCAGG
      TCCGGACCGT TGAAGGAGGT CAGGTCCGGA CTCGGGTGTC GGGGAGGGCT CCTCAGGAAG TCCAAACCCC TCCTCTTCTG GTGGGGGTCG GTCTTCGTCC
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2701  AGCCCATTGA CAAGGAGCTG TACCCCCTGG CCTCCCTGAG GTCCCTGTTT GGCAACGACC CCTCCTCCCA GTAAAATAAA GCCCGGGCAG ATCTGCTGTG
      TCGGGTAACT GTTCCTCGAC ATGGGGGACC GGAGGGACTC CAGGGACAAA CCGTTGCTGG GGAGGAGGGT CATTTTATTT CGGGCCCGTC TAGACGACAC
2801  CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA
      GGAAGATCAA CGGTCGGTAG ACAACAAACG GGGAGGGGGC ACGGAAGGAA CTGGGACCTT CCACGGTGAG GGTGACAGGA AAGGATTATT TTACTCCTTT
                                                                                                          SphI
2901  TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA GGCATGCTGG
      AACGTAGCGT AACAGACTCA TCCACAGTAA GATAAGACCC CCCACCCCAC CCCGTCCTGT CGTTCCCCCT CCTAACCCTT CTGTTATCGT CCGTACGACC
                          PvuI
                               AscI
3001  GGATGCGGTG GGCTCTATGG CCGATCGGCG CGCCGTACTG AAATGTGTGG GCGTGGCTTA AGGGTGGGAA AGAATATATA AGGTGGGGGT CTTATGTAGT
      CCTACGCCAC CCGAGATACC GGCTAGCCGC GCGGCATGAC TTTACACACC CGCACCGAAT TCCCACCCTT TCTTATATAT TCCACCCCCA GAATACATCA
                                                                                               SphI
3101  TTTGTATCTG TTTTGCAGCA GCCGCCGCCG CCATGAGCAC CAACTCGTTT GATGGAAGCA TTGTGAGCTC ATATTTGACA ACGCGCATGC CCCCATGGGC
      AAACATAGAC AAAACGTCGT CGGCGGCGGC GGTACTCGTG GTTGAGCAAA CTACCTTCGT AACACTCGAG TATAAACTGT TGCGCGTACG GGGGTACCCG
3201  CGGGGTGCGT CAGAATGTGA TGGGCTCCAG CATTGATGGT CGCCCCGTCC TGCCCGCAAA CTCTACTACC TTGACCTACG AGACCGTGTC TGGAACGCCG
      GCCCCACGCA GTCTTACACT ACCCGAGGTC GTAACTACCA GCGGGGCAGG ACGGGCGTTT GAGATGATGG AACTGGATGC TCTGGCACAG ACCTTGCGGC
```

Figure 15B

pMRKAd5gag HER6R2

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            PstI                    PstI
3301  TTGGAGACTG CAGCCTCCGC CGCCGCTTCA GCCGCTGCAG CCACCGCCCG CGGGATTGTG ACTGACTTTG CTTTCCTGAG CCCGCTTGCA AACAGTGCAG
      AACCTCTGAC GTCGGAGGCG GCGGCGAAGT CGGCGACGTC GGTGGCGGGC GCCCTAACAC TGACTGAAAC GAAAGGACTC GGGCGAACGT TTGTCACGTC
3401  CTTCCCGTTC ATCCGCCCGC GATGACAAGT TGACGGCTCT TTTGGCACAA TTGGATTCTT TGACCCGGGA ACTTAATGTC GTTTCTCAGC AGCTGTTGGA
      GAAGGGCAAG TAGGCGGGCG CTACTGTTCA ACTGCCGAGA AAACCGTGTT AACCTAAGAA ACTGGGCCCT TGAATTACAG CAAAGAGTCG TCGACAACCT
3501  TCTGCGCCAG CAGGTTTCTG CCCTGAAGGC TTCCTCCCCT CCCAATGCGG TTTAAAACAT AAATAAAAAA CCAGACTCTG TTTGGATTTG GATCAAGCAA
      AGACGCGGTC GTCCAAAGAC GGGACTTCCG AAGGAGGGGA GGGTTACGCC AAATTTTGTA TTTATTTTTT GGTCTGAGAC AAACCTAAAC CTAGTTCGTT
3601  GTGTCTTGCT GTCTTTATTT AGGGGTTTTG CGCGCGCGGT AGGCCCGGGA CCAGCGGTCT CGGTCGTTGA GGGTCCTGTG TATTTTTTCC AGGACGTGGT
      CACAGAACGA CAGAAATAAA TCCCCAAAAC GCGCGCGCCA TCCGGGCCCT GGTCGCCAGA GCCAGCAACT CCCAGGACAC ATAAAAAAGG TCCTGCACCA
                                                                              PstI
3701  AAAGGTGACT CTGGATGTTC AGATACATGG GCATAAGCCC GTCTCTGGGG TGGAGGTAGC ACCACTGCAG AGCTTCATGC TGCGGGGTGG TGTTGTAGAT
      TTTCCACTGA GACCTACAAG TCTATGTACC CGTATTCGGG CAGAGACCCC ACCTCCATCG TGGTGACGTC TCGAAGTACG ACGCCCCACC ACAACATCTA
3801  GATCCAGTCG TAGCAGGAGC GCTGGGCGTG GTGCCTAAAA ATGTCTTTCA GTAGCAAGCT GATTGCCAGG GGCAGGCCCT TGGTGTAAGT GTTTACAAAG
      CTAGGTCAGC ATCGTCCTCG CGACCCGCAC CACGGATTTT TACAGAAAGT CATCGTTCGA CTAACGGTCC CCGTCCGGGA ACCACATTCA CAAATGTTTC
3901  CGGTTAAGCT GGGATGGGTG CATACGTGGG GATATGAGAT GCATCTTGGA CTGTATTTTT AGGTTGGCTA TGTTCCCAGC CATATCCCTC CGGGGATTCA
      GCCAATTCGA CCCTACCCAC GTATGCACCC CTATACTCTA CGTAGAACCT GACATAAAAA TCCAACCGAT ACAAGGGTCG GTATAGGGAG GCCCCTAAGT
4001  TGTTGTGCAG AACCACCAGC ACAGTGTATC CGGTGCACTT GGGAAATTTG TCATGTAGCT TAGAAGGAAA TGCGTGGAAG AACTTGGAGA CGCCCTTGTG
      ACAACACGTC TTGGTGGTCG TGTCACATAG GCCACGTGAA CCCTTTAAAC AGTACATCGA ATCTTCCTTT ACGCACCTTC TTGAACCTCT GCGGGAACAC
4101  ACCTCCAAGA TTTTCCATGC ATTCGTCCAT AATGATGGCA ATGGGCCCAC GGGCGGCGGC CTGGGCGAAG ATATTTCTGG GATCACTAAC GTCATAGTTG
      TGGAGGTTCT AAAAGGTACG TAAGCAGGTA TTACTACCGT TACCCGGGTG CCCGCCGCCG GACCCGCTTC TATAAAGACC CTAGTGATTG CAGTATCAAC
4201  TGTTCCAGGA TGAGATCGTC ATAGGCCATT TTTACAAAGC GCGGGCGGAG GGTGCCAGAC TGCGGTATAA TGGTTCCATC CGGCCCAGGG GCGTAGTTAC
      ACAAGGTCCT ACTCTAGCAG TATCCGGTAA AAATGTTTCG CGCCCGCCTC CCACGGTCTG ACGCCATATT ACCAAGGTAG GCCGGGTCCC CGCATCAATG
4301  CCTCACAGAT TTGCATTTCC CACGCTTTGA GTTCAGATGG GGGGATCATG TCTACCTGCG GGGCGATGAA GAAAACGGTT TCCGGGGTAG GGGAGATCAG
      GGAGTGTCTA AACGTAAAGG GTGCGAAACT CAAGTCTACC CCCCTAGTAC AGATGGACGC CCCGCTACTT CTTTTGCCAA AGGCCCCATC CCCTCTAGTC
                                                                                                          PstI
4401  CTGGGAAGAA AGCAGGTTCC TGAGCAGCTG CGACTTACCG CAGCCGGTGG GCCCGTAAAT CACACCTATT ACCGGCTGCA ACTGGTAGTT AAGAGAGCTG
      GACCCTTCTT TCGTCCAAGG ACTCGTCGAC GCTGAATGGC GTCGGCCACC CGGGCATTTA GTGTGGATAA TGGCCGACGT TGACCATCAA TTCTCTCGAC
      PstI
4501  CAGCTGCCGT CATCCCTGAG CAGGGGGGCC ACTTCGTTAA GCATGTCCCT GACTCGCATG TTTTCCCTGA CCAAATCCGC CAGAAGGCGC TCGCCGCCCA
      GTCGACGGCA GTAGGGACTC GTCCCCCCGG TGAAGCAATT CGTACAGGGA CTGAGCGTAC AAAAGGGACT GGTTTAGGCG GTCTTCCGCG AGCGGCGGGT
                                                                  SphI
4601  GCGATAGCAG TTCTTGCAAG GAAGCAAAGT TTTTCAACGG TTTGAGACCG TCCGCCGTAG GCATGCTTTT GAGCGTTTGA CCAAGCAGTT CCAGGCGGTC
      CGCTATCGTC AAGAACGTTC CTTCGTTTCA AAAAGTTGCC AAACTCTGGC AGGCGGCATC CGTACGAAAA CTCGCAAACT GGTTCGTCAA GGTCCGCCAG
4701  CCACAGCTCG GTCACCTGCT CTACGGCATC TCGATCCAGC ATATCTCCTC GTTTCGCGGG TTGGGGCGGC TTTCGCTGTA CGGCAGTAGT CGGTGCTCGT
      GGTGTCGAGC CAGTGGACGA GATGCCGTAG AGCTAGGTCG TATAGAGGAG CAAAGCGCCC AACCCCGCCG AAAGCGACAT GCCGTCATCA GCCACGAGCA
4801  CCAGACGGGC CAGGGTCATG TCTTTCCACG GGCGCAGGGT CCTCGTCAGC GTAGTCTGGG TCACGGTGAA GGGGTGCGCT CCGGGCTGCG CGCTGGCCAA
      GGTCTGCCCG GTCCCAGTAC AGAAAGGTGC CCGCGTCCCA GGAGCAGTCG CATCAGACCC AGTGCCACTT CCCCACGCGA GGCCCGACGC GCGACCGGTT
```

Figure 15C

pMRKAd5gag MER682

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4901  GGTGCGCTTG AGGCTGGTCC TGCTGGTGCT GAAGCGCTGC CGGTCTTCGC CCTGCGCGTC GGCCAGGTAG CATTTGACCA TGGTGTCATA GTCCAGCCCC
      CCACGCGAAC TCCGACCAGG ACGACCACGA CTTCGCGACG GCCAGAAGCG GGACGCGCAG CCGGTCCATC GTAAACTGGT ACCACAGTAT CAGGTCGGGG
5001  TCCGCGGCGT GGCCCTTGGC GCGCAGCTTG CCCTTGGAGG AGGCGCCGCA CTAGGGGCAG TGCAGACTTT TGAGGGCGTA GAGCTTGGGC GCGAGAAATA
      AGGCGCCGCA CCGGGAACCG CGCGTCGAAC GGGAACCTCC TCCGCGGCGT GATCCCCGTC ACGTCTGAAA ACTCCCGCAT CTCGAACCCG CGCTCTTTAT
5101  CCGATTCCGG GGAGTAGGCA TCCGCGCCGC AGGCCCCGCA GACGGTCTCG CATTCCACGA GCCAGGTGAG CTCTGGCCGT TCGGGGTCAA AAACCAGGTT
      GGCTAAGGCC CCTCATCCGT AGGCGCGGCG TCCGGGGCGT CTGCCAGAGC GTAAGGTGCT CGGTCCACTC GAGACCGGCA AGCCCCAGTT TTTGGTCCAA
                                                                                                   Bst1107I
5201  TCCCCCATGC TTTTTGATGC GTTTCTTACC TCTGGTTTCC ATGAGCCGGT GTCCACGCTC GGTGACGAAA AGGCTGTCCG TGTCCCCGTA TACAGACTTG
      AGGGGGTACG AAAAACTACG CAAAGAATGG AGACCAAAGG TACTCGGCCA CAGGTGCGAG CCACTGCTTT TCCGACAGGC ACAGGGGCAT ATGTCTGAAC
                 XhoI
5301  AGAGGCCTGT CCTCGAGCGG TGTTCCGCGG TCCTCCTCGT ATAGAAACTC GGACCACTCT GAGACAAAGG CTCGCGTCCA GGCCAGCACG AAGGAGGCTA
      TCTCCGGACA GGAGCTCGCC ACAAGGCGCC AGGAGGAGCA TATCTTTGAG CCTGGTGAGA CTCTGTTTCC GAGCGCAGGT CCGGTCGTGC TTCCTCCGAT
5401  AGTGGGAGGG GTAGCGGTCG TTGTCCACTA GGGGGTCCAC TCGCTCCAGG GTGTGAAGAC ACATGTCGCC CTCTTCGGCA TCAAGGAAGG TGATTGGTTT
      TCACCCTCCC CATCGCCAGC AACAGGTGAT CCCCCAGGTG AGCGAGGTCC CACACTTCTG TGTACAGCGG GAGAAGCCGT AGTTCCTTCC ACTAACCAAA
5501  GTAGGTGTAG GCCACGTGAC CGGGTGTTCC TGAAGGGGGG CTATAAAAGG GGGTGGGGGC GCGTTCGTCC TCACTCTCTT CCGCATCGCT GTCTGCGAGG
      CATCCACATC CGGTGCACTG GCCCACAAGG ACTTCCCCCC GATATTTTCC CCCACCCCCG CGCAAGCAGG AGTGAGAGAA GGCGTAGCGA CAGACGCTCC
5601  GCCAGCTGTT GGGGTGAGTA CTCCCTCTGA AAAGCGGGCA TGACTTCTGC GCTAAGATTG TCAGTTTCCA AAAACGAGGA GGATTTGATA TTCACCTGGC
      CGGTCGACAA CCCCACTCAT GAGGGAGACT TTTCGCCCGT ACTGAAGACG CGATTCTAAC AGTCAAAGGT TTTTGCTCCT CCTAAACTAT AAGTGGACCG
                                                                             HindIII
5701  CCGCGGTGAT GCCTTTGAGG GTGGCCGCAT CCATCTGGTC AGAAAAGACA ATCTTTTTGT TGTCAAGCTT GGTGGCAAAC GACCCGTAGA GGGCGTTGGA
      GGCGCCACTA CGGAAACTCC CACCGGCGTA GGTAGACCAG TCTTTTCTGT TAGAAAAACA ACAGTTCGAA CCACCGTTTG CTGGGCATCT CCCGCAACCT
                                                PvuI
5801  CAGCAACTTG GCGATGGAGC GCAGGGTTTG GTTTTTGTCG CGATCGGCGC GCTCCTTGGC CGCGATGTTT AGCTGCACGT ATTCGCGCGC AACGCACCGC
      GTCGTTGAAC CGCTACCTCG CGTCCCAAAC CAAAAACAGC GCTAGCCGCG CGAGGAACCG GCGCTACAAA TCGACGTGCA TAAGCGCGCG TTGCGTGGCG
5901  CATTCGGGAA AGACGGTGGT GCGCTCGTCG GGCACCAGGT GCACGCGCCA ACCGCGGTTG TGCAGGGTGA CAAGGTCAAC GCTGGTGGCT ACCTCTCCGC
      GTAAGCCCTT TCTGCCACCA CGCGAGCAGC CCGTGGTCCA CGTGCGCGGT TGGCGCCAAC ACGTCCCACT GTTCCAGTTG CGACCACCGA TGGAGAGGCG
6001  GTAGGCGCTC GTTGGTCCAG CAGAGGCGGC CGCCCTTGCG CGAGCAGAAT GGCGGTAGGG GGTCTAGCTG CGTCTCGTCC GGGGGGTCTG CGTCCACGGT
      CATCCGCGAG CAACCAGGTC GTCTCCGCCG GCGGGAACGC GCTCGTCTTA CCGCCATCCC CCAGATCGAC GCAGAGCAGG CCCCCCAGAC GCAGGTGCCA
6101  AAAGACCCCG GGCAGCAGGC GCGCGTCGAA GTAGTCTATC TTGCATCCTT GCAAGTCTAG CGCCTGCTGC CATGCGCGGG CGGCAAGCGC GCGCTCGTAT
      TTTCTGGGGC CCGTCGTCCG CGCGCAGCTT CATCAGATAG AACGTAGGAA CGTTCAGATC GCGGACGACG GTACGCGCCC GCCGTTCGCG CGCGAGCATA
6201  GGGTTGAGTG GGGGACCCCA TGGCATGGGG TGGGTGAGCG CGGAGGCGTA CATGCCGCAA ATGTCGTAAA CGTAGAGGGG CTCTCTGAGT ATTCCAAGAT
      CCCAACTCAC CCCCTGGGGT ACCGTACCCC ACCCACTCGC GCCTCCGCAT GTACGGCGTT TACAGCATTT GCATCTCCCC GAGAGACTCA TAAGGTTCTA
6301  ATGTAGGGTA GCATCTTCCA CCGCGGATGC TGGCGCGCAC GTAATCGTAT AGTTCGTGCG AGGGAGCGAG GAGGTCGGGA CCGAGGTTGC TACGGGCGGG
      TACATCCCAT CGTAGAAGGT GGCGCCTACG ACCGCGCGTG CATTAGCATA TCAAGCACGC TCCCTCGCTC CTCCAGCCCT GGCTCCAACG ATGCCCGCCC
6401  CTGCTCTGCT CGGAAGACTA TCTGCCTGAA GATGGCATGT GAGTTGGATG ATATGGTTGG ACGCTGGAAG ACGTTGAAGC TGGCGTCTGT GAGACCTACC
      GACGAGACGA GCCTTCTGAT AGACGGACTT CTACCGTACA CTCAACCTAC TATACCAACC TGCGACCTTC TGCAACTTCG ACCGCAGACA CTCTGGATGG
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Figure 15D

pMRKAd5gag MER6R2

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6501  GCGTCACGCA CGAAGGAGGC GTAGGAGTCG CGCAGCTTGT TGACCAGCTC GGCGGTGACC TGCACGTCTA GGGCGCAGTA GTCCAGGGTT TCCTTGATGA
      CGCAGTGCGT GCTTCCTCCG CATCCTCAGC GCGTCGAACA ACTGGTCGAG CCGCCACTGG ACGTGCAGAT CCCGCGTCAT CAGGTCCCAA AGGAACTACT
6601  TGTCATACTT ATCCTGTCCC TTTTTTTTCC ACAGCTCGCG GTTGAGGACA AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC
      ACAGTATGAA TAGGACAGGG AAAAAAAAGG TGTCGAGCGC CAACTCCTGT TTGAGAAGCG CCAGAAAGGT CATGAGAACC TAGCCTTTGG GCAGCCGGAG
6701  CGAACGGTAA GAGCCTAGCA TGTAGAACTG GTTGACGGCC TGGTAGGCGC AGCATCCCTT TTCTACGGGT AGCGCGTATG CCTGCGCGGC CTTCCGGAGC
      GCTTGCCATT CTCGGATCGT ACATCTTGAC CAACTGCCGG ACCATCCGCG TCGTAGGGAA AAGATGCCCA TCGCGCATAC GGACGCGCCG GAAGGCCTCG
6801  GAGGTGTGGG TGAGCGCAAA GGTGTCCCTG ACCATGACTT TGAGGTACTG GTATTTGAAG TCAGTGTCGT CGCATCCGCC CTGCTCCCAG AGCAAAAAGT
      CTCCACACCC ACTCGCGTTT CCACAGGGAC TGGTACTGAA ACTCCATGAC CATAAACTTC AGTCACAGCA GCGTAGGCGG GACGAGGGTC TCGTTTTTCA
6901  CCGTGCGCTT TTTGGAACGC GGATTTGGCA GGGCGAAGGT GACATCGTTG AAGAGTATCT TTCCCGCGCG AGGCATAAAG TTGCGTGTGA TGCGGAAGGG
      GGCACGCGAA AAACCTTGCG CCTAAACCGT CCCGCTTCCA CTGTAGCAAC TTCTCATAGA AAGGGCGCGC TCCGTATTTC AACGCACACT ACGCCTTCCC
7001  TCCCGGCACC TCGGAACGGT TGTTAATTAC CTGGGCGGCG AGCACGATCT CGTCAAAGCC GTTGATGTTG TGGCCCACAA TGTAAAGTTC CAAGAAGCGC
      AGGGCCGTGG AGCCTTGCCA ACAATTAATG GACCCGCCGC TCGTGCTAGA GCAGTTTCGG CAACTACAAC ACCGGGTGTT ACATTTCAAG GTTCTTCGCG
7101  GGGATGCCCT TGATGGAAGG CAATTTTTTA AGTTCCTCGT AGGTGAGCTC TTCAGGGGAG CTGAGCCCGT GCTCTGAAAG GGCCCAGTCT GCAAGATGAG
      CCCTACGGGA ACTACCTTCC GTTAAAAAAT TCAAGGAGCA TCCACTCGAG AAGTCCCCTC GACTCGGGCA CGAGACTTTC CCGGGTCAGA CGTTCTACTC
7201  GGTTGGAAGC GACGAATGAG CTCCACAGGT CACGGGCCAT TAGCATTTGC AGGTGGTCGC GAAAGGTCCT AAACTGGCGA CCTATGGCCA TTTTTTCTGG
      CCAACCTTCG CTGCTTACTC GAGGTGTCCA GTGCCCGGTA ATCGTAAACG TCCACCAGCG CTTTCCAGGA TTTGACCGCT GGATACCGGT AAAAAAGACC
7301  GGTGATGCAG TAGAAGGTAA GCGGGTCTTG TTCCCAGCGG TCCCATCCAA GGTTCGCGGC TAGGTCTCGC GCGGCAGTCA CTAGAGGCTC ATCTCCGCCG
      CCACTACGTC ATCTTCCATT CGCCCAGAAC AAGGGTCGCC AGGGTAGGTT CCAAGCGCCG ATCCAGAGCG CGCCGTCAGT GATCTCCGAG TAGAGGCGGC
7401  AACTTCATGA CCAGCATGAA GGGCACGAGC TGCTTCCCAA AGGCCCCCAT CCAAGTATAG GTCTCTACAT CGTAGGTGAC AAAGAGACGC TCGGTGCGAG
      TTGAAGTACT GGTCGTACTT CCCGTGCTCG ACGAAGGGTT TCCGGGGGTA GGTTCATATC CAGAGATGTA GCATCCACTG TTTCTCTGCG AGCCACGCTC
                 PvuI
7501  GATGCGAGCC GATCGGGAAG AACTGGATCT CCCGCCACCA ATTGGAGGAG TGGCTATTGA TGTGGTGAAA GTAGAAGTCC CTGCGACGGG CCGAACACTC
      CTACGCTCGG CTAGCCCTTC TTGACCTAGA GGGCGGTGGT TAACCTCCTC ACCGATAACT ACACCACTTT CATCTTCAGG GACGCTGCCC GGCTTGTGAG
7601  GTGCTGGCTT TTGTAAAAAC GTGCGCAGTA CTGGCAGCGG TGCACGGGCT GTACATCCTG CACGAGGTTG ACCTGACGAC CGCGCACAAG GAAGCAGAGT
      CACGACCGAA AACATTTTTG CACGCGTCAT GACCGTCGCC ACGTGCCCGA CATGTAGGAC GTGCTCCAAC TGGACTGCTG GCGCGTGTTC CTTCGTCTCA
                                                                                      XhoI
7701  GGGAATTTGA GCCCCTCGCC TGGCGGGTTT GGCTGGTGGT CTTCTACTTC GGCTGCTTGT CCTTGACCGT CTGGCTGCTC GAGGGGAGTT ACGGTGGATC
      CCCTTAAACT CGGGGAGCGG ACCGCCCAAA CCGACCACCA GAAGATGAAG CCGACGAACA GGAACTGGCA GACCGACGAG CTCCCCTCAA TGCCACCTAG
7801  GGACCACCAC GCCGCGCGAG CCCAAAGTCC AGATGTCCGC GCGCGGCGGT CGGAGCTTGA TGACAACATC GCGCAGATGG GAGCTGTCCA TGGTCTGGAG
      CCTGGTGGTG CGGCGCGCTC GGGTTTCAGG TCTACAGGCG CGCGCCGCCA GCCTCGAACT ACTGTTGTAG CGCGTCTACC CTCGACAGGT ACCAGACCTC
                                        PstI
7901  CTCCCGCGGC GTCAGGTCAG GCGGGAGCTC CTGCAGGTTT ACCTCGCATA GACGGGTCAG GGCGCGGGCT AGATCCAGGT GATACCTAAT TTCCAGGGGC
      GAGGGCGCCG CAGTCCAGTC CGCCCTCGAG GACGTCCAAA TGGAGCGTAT CTGCCCAGTC CCGCGCCCGA TCTAGGTCCA CTATGGATTA AAGGTCCCCG
                                                                 KpnI
8001  TGGTTGGTGG CGGCGTCGAT GGCTTGCAAG AGGCCGCATC CCCGCGGCGC GACTACGGTA CCGCGCGGCG GGCGGTGGGC CGCGGGGGTG TCCTTGGATG
      ACCAACCACC GCCGCAGCTA CCGAACGTTC TCCGGCGTAG GGGCGCCGCG CTGATGCCAT GGCGCGCCGC CCGCCACCCG GCGCCCCCAC AGGAACCTAC
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Figure 15E

pNRKAd5gag NER682

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8101  ATGCATCTAA AACCGGTGAC GCGGGCGAGC CCCCGGAGGT AGGGGGGGCT CCGGACCCGC CGGGAGAGGG GGCAGGGGCA CGTCGGCGCC GCGCGCGGGC
      TACGTAGATT TTGGCCACTG CGCCCGCTCG GGGGCCTCCA TCCCCCCCGA GGCCTGGGCG GCCCTCTCCC CCGTCCCCGT GCAGCCGCGG CGCGCGCCCG
8201  AGGAGCTGGT GCTGCGCGCG TAGGTTGCTG GCGAACGCGA CGACGCGCCC GTTGATCTCC TGAATCTGGC GCCTCTGCGT GAAGACGACG GGCCGGGTGA
      TCCTCGACCA CGACGCGCGC ATCCAACGAC CGCTTGCGCT GCTGCGCGGG CAACTAGAGG ACTTAGACCG CGGAGACGCA CTTCTGCTGC CCGGCCCACT
8301  GCTTGAACCT GAAAGAGAGT TCGACAGAAT CAATTTCGGT GTCGTTGACG GCGGCCTGGC GCAAAATCTC CTGCACGTCT CCTGAGTTGT CTTGATAGG
      CGAACTTGGA CTTTCTCTCA AGCTGTCTTA GTTAAAGCCA CAGCAACTGC CGCCGGACCG CGTTTTAGAG GACGTGCAGA GGACTCAACA GAACTATCC
                                       BglII
8401  GATCTCGGCC ATGAACTGCT CGATCTCTTC CTCCTGGAGA TCTCCGCGTC CGGCTCGCTC CACGGTGGCG GCGAGGTCGT TGGAAATGCG GGCCATGA
      CTAGAGCCGG TACTTGACGA GCTAGAGAAG GAGGACCTCT AGAGGCGCAG GCCGAGCGAG GTGCCACCGC CGCTCCAGCA ACCTTTACGC CCGGTACT
8501  TGCGAGAAGG CGTTGAGGCC TCCCTCGTTC CAGACGCGGC TGTAGACCAC GCCCCCTTCG GCATCGCGGG CGCGCATGAC CACCTGCGCG AGATTGAGC
      ACGCTCTTCC GCAACTCCGG AGGGAGCAAG GTCTGCGCCG ACATCTGGTG CGGGGGAAGC CGTAGCGCCC GCGCGTACTG GTGGACGCGC TCTAACTCG
8601  CCACGTGCCG GGCGAAGACG GCGTAGTTTC GCAGGCGCTG AAAGAGGTAG TTGAGGGTGG TGGCGGTGTG TTCTGCCACG AAGAAGTACA TAACCCAGC
      GGTGCACGGC CCGCTTCTGC CGCATCAAAG CGTCCGCGAC TTTCTCCATC AACTCCCACC ACCGCCACAC AAGACGGTGC TTCTTCATGT ATTGGGTCG
                       EcoRV
8701  TCGCAACGTG GATTCGTTGA TATCCCCCAA GGCCTCAAGG CGCTCCATGG CCTCGTAGAA GTCCACGGCG AAGTTGAAAA ACTGGGAGTT GCGCGCCGAC
      AGCGTTGCAC CTAAGCAACT ATAGGGGGTT CCGGAGTTCC GCGAGGTACC GGAGCATCTT CAGGTGCCGC TTCAACTTTT TGACCCTCAA CGCGCGGCTG
8801  ACGGTTAACT CCTCCTCCAG AAGACGGATG AGCTCGGCGA CAGTGTCGCG CACCTCGCGC TCAAAGGCTA CAGGGGCCTC TTCTTCTTCT TCAATCTCCT
      TGCCAATTGA GGAGGAGGTC TTCTGCCTAC TCGAGCCGCT GTCACAGCGC GTGGAGCGCG AGTTTCCGAT GTCCCCGGAG AAGAAGAAGA AGTTAGAGGA
                                                                                                SalI
8901  CTTCCATAAG GGCCTCCCCT TCTTCTTCTT CTGGCGGCGG TGGGGGAGGG GGGACACGGC GGCGACGACG GCGCACCGGG AGGCGGTCGA CAAAGCGCTC
      GAAGGTATTC CCGGAGGGGA AGAAGAAGAA GACCGCCGCC ACCCCCTCCC CCCTGTGCCG CCGCTGCTGC CGCGTGGCCC TCCGCCAGCT GTTTCGCGAG
9001  GATCATCTCC CCGCGGCGAC GGCGCATGGT CTCGGTGACG GCGCGGCCGT TCTCGCGGGG GCGCAGTTGG AAGACGCCGC CCGTCATGTC CCGGTTATGG
      CTAGTAGAGG GGCGCCGCTG CCGCGTACCA GAGCCACTGC CGCGCCGGCA AGAGCGCCCC CGCGTCAACC TTCTGCGGCG GGCAGTACAG GGCCAATACC
9101  GTTGGCGGGG GGCTGCCATG CGGCAGGGAT ACGGCGCTAA CGATGCATCT CAACAATTGT TGTGTAGGTA CTCCGCCGCC GAGGGACCTG AGCGAGTCC
      CAACCGCCCC CCGACGGTAC GCCGTCCCTA TGCCGCGATT GCTACGTAGA GTTGTTAACA ACACATCCAT GAGGCGGCGG CTCCCTGGAC TCGCTCAGG
                       XhoI
9201  CATCGACCGG ATCGGAAAAC CTCTCGAGAA AGGCGTCTAA CCAGTCACAG TCGCAAGGTA GGCTGAGCAC CGTGGCGGGC GGCAGCGGGC GGCGGTCGG
      GTAGCTGGCC TAGCCTTTTG GAGAGCTCTT TCCGCAGATT GGTCAGTGTC AGCGTTCCAT CCGACTCGTG GCACCGCCCG CCGTCGCCCG CCGCCAGCCC
                                                                         SalI
9301  GTTGTTTCTG GCGGAGGTGC TGCTGATGAT GTAATTAAAG TAGGCGGTCT TGAGACGGCG GATGGTCGAC AGAAGCACCA TGTCCTTGGG TCCGGCCTGC
      CAACAAAGAC CGCCTCCACG ACGACTACTA CATTAATTTC ATCCGCCAGA ACTCTGCCGC CTACCAGCTG TCTTCGTGGT ACAGGAACCC AGGCCGGACG
9401  TGAATGCGCA GGCGGTCGGC CATGCCCCAG GCTTCGTTTT GACATCGGCG CAGGTCTTTG TAGTAGTCTT GCATGAGCCT TTCTACCGGC ACTTCTTCT
      ACTTACGCGT CCGCCAGCCG GTACGGGGTC CGAAGCAAAA CTGTAGCCGC GTCCAGAAAC ATCATCAGAA CGTACTCGGA AAGATGGCCG TGAAGAAGA
9501  CTCCTTCCTC TTGTCCTGCA TCTCTTGCAT CTATCGCTGC GGCGGCGGCG GAGTTTGGCC GTAGGTGGCG CCCTCTTCCT CCCATGCGTG TGACCCCGA
      GAGGAAGGAG AACAGGACGT AGAGAACGTA GATAGCGACG CCGCCGCCGC CTCAAACCGG CATCCACCGC GGGAGAAGGA GGGTACGCAC ACTGGGGCT
9601  GCCCCTCATC GGCTGAAGCA GGGCTAGGTC GGCGACAACG CGCTCGGCTA ATATGGCCTG CTGCACCTGC GTGAGGGTAG ACTGGAAGTC ATCCATGTC
      CGGGGAGTAG CCGACTTCGT CCCGATCCAG CCGCTGTTGC GCGAGCCGAT TATACCGGAC GACGTGGACG CACTCCCATC TGACCTTCAG TAGGTACAG
```

Figure 15F

pMRKAd5gag MERG82

9701 ACAAAGCGGT GGTATGCGCC CGTGTTGATG GTGTAAGTGC AGTTGGCCAT AACGGACCAG TTAACGGTCT GGTGACCCGG CTGCGAGAGC TCGGTGTACC
TGTTTCGCCA CCATACGCGG GCACAACTAC CACATTCACG TCAACCGGTA TTGCCTGGTC AATTGCCAGA CCACTGGGCC GACGCTCTCG AGCCACATGG

XhoI

9801 TGAGACGCGA GTAAGCCCTC GAGTCAAATA CGTAGTCGTT GCAAGTCCGC ACCAGGTACT GGTATCCCAC CAAAAAGTGC GGCGGCGGCT GGCGGTAGAG
ACTCTGCGCT CATTCGGGAG CTCAGTTTAT GCATCAGCAA CGTTCAGGCG TGGTCCATGA CCATAGGGTG GTTTTTCACG CCGCCGCCGA CCGCCATCTC

BglII EcoRV

9901 GGGCCAGCGT AGGGTGGCCG GGGCTCCGGG GGCGAGATCT TCCAACATAA GGCGATGATA TCCGTAGATG TACCTGGACA TCCAGGTGAT GCCGGCGGCG
CCCGGTCGCA TCCCACCGGC CCCGAGGCCC CCGCTCTAGA AGGTTGTATT CCGCTACTAT AGGCATCTAC ATGGACCTGT AGGTCCACTA CGGCCGCCGC

10001 GTGGTGGAGG CGCGCGGAAA GTCGCGGACG CGGTTCCAGA TGTTGCGCAG CGGCAAAAAG TGCTCCATGG TCGGGACGCT CTGGCCGGTC AGGCGCGCGC
CACCACCTCC GCGCGCCTTT CAGCGCCTGC GCCAAGGTCT ACAACGCGTC GCCGTTTTTC ACGAGGTACC AGCCCTGCGA GACCGGCCAG TCCGCGCGCG

XbaI

10101 AATCGTTGAC GCTCTAGACC GTGCAAAAGG AGAGCCTGTA AGCGGGCACT CTTCCGTGGT CTGGTGGATA AATTCGCAAG GGTATCATGG CGGACGACCG
TTAGCAACTG CGAGATCTGG CACGTTTTCC TCTCGGACAT TCGCCCGTGA GAAGGCACCA GACCACCTAT TTAAGCGTTC CCATAGTACC GCCTGCTGGC

10201 GGGTTCGAGC CCCGTATCCG GCCGTCCGCC GTGATCCATG CGGTTACCGC CCGCGTGTCG AACCCAGGTG TGCGACGTCA GACAACGGGG GAGTGCTCCT
CCCAAGCTCG GGGCATAGGC CGGCAGGCGG CACTAGGTAC GCCAATGGCG GGCGCACAGC TTGGGTCCAC ACGCTGCAGT CTGTTGCCCC CTCACGAGGA

10301 TTTGGCTTCC TTCCAGGCGC GGCGGCTGCT GCGCTAGCTT TTTTGGCCAC TGGCCGCGCG CAGCGTAAGC GGTTAGGCTG GAAAGCGAAA GCATTAAGTG
AAACCGAAGG AAGGTCCGCG CCGCCGACGA CGCGATCGAA AAAACCGGTG ACCGGCGCGC GTCGCATTCG CCAATCCGAC CTTTCGCTTT CGTAATTCAC

10401 GCTCGCTCCC TGTAGCCGGA GGGTTATTTT CCAAGGGTTG AGTCGCGGGA CCCCCGGTTC GAGTCTCGGA CCGGCCGGAC TGCGGCGAAC GGGGGTTTGC
CGAGCGAGGG ACATCGGCCT CCCAATAAAA GGTTCCCAAC TCAGCGCCCT GGGGGCCAAG CTCAGAGCCT GGCCGGCCTG ACGCCGCTTG CCCCCAAACG

10501 CTCCCCGTCA TGCAAGACCC CGCTTGCAAA TTCCTCCGGA AACAGGGACG AGCCCCTTTT TTGCTTTTCC CAGATGCATC CGGTGCTGCG GCAGATGCG
GAGGGGCAGT ACGTTCTGGG GCGAACGTTT AAGGAGGCCT TTGTCCCTGC TCGGGGAAAA AACGAAAAGG GTCTACGTAG GCCACGACGC CGTCTACGC

10601 CCCCCTCCTC AGCAGCGGCA AGAGCAAGAG CAGCGGCAGA CATGCAGGGC ACCCTCCCCT CCTCCTACCG CGTCAGGAGG GGCGACATCC GCGGTTGACG
GGGGGAGGAG TCGTCGCCGT TCTCGTTCTC GTCGCCGTCT GTACGTCCCG TGGGAGGGGA GGAGGATGGC GCAGTCCTCC CCGCTGTAGG CGCCAACTGC

10701 CGGCAGCAGA TGGTGATTAC GAACCCCCGC GGCGCCGGGC CCGGCACTAC CTGGACTTGG AGGAGGGCGA GGGCCTGGCG CGGCTAGGAG CGCCCTCTCC
GCCGTCGTCT ACCACTAATG CTTGGGGGCG CCGCGGCCCG GGCCGTGATG GACCTGAACC TCCTCCCGCT CCCGGACCGC GCCGATCCTC GCGGGAGAGG

10801 TGAGCGGCAC CCAAGGGTGC AGCTGAAGCG TGATACGCGT GAGGCGTACG TGCCGCGGCA GAACCTGTTT CGCGACCGCG AGGGAGAGGA GCCCGAGGAG
ACTCGCCGTG GGTTCCCACG TCGACTTCGC ACTATGCGCA CTCCGCATGC ACGGCGCCGT CTTGGACAAA GCGCTGGCGC TCCCTCTCCT CGGGCTCCTC

10901 ATGCGGGATC GAAAGTTCCA CGCAGGGCGC GAGCTGCGGC ATGGCCTGAA TCGCGAGCGG TTGCTGCGCG AGGAGGACTT TGAGCCCGAC GCGCGAACCG
TACGCCCTAG CTTTCAAGGT GCGTCCCGCG CTCGACGCCG TACCGGACTT AGCGCTCGCC AACGACGCGC TCCTCCTGAA ACTCGGGCTG CGCGCTTGGC

HindIII

11001 GGATTAGTCC CGCGCGCGCA CACGTGGCGG CCGCCGACCT GGTAACCGCA TACGAGCAGA CGGTGAACCA GGAGATTAAC TTTCAAAAAA GCTTTAACAA
CCTAATCAGG GCGCGCGCGT GTGCACCGCC GGCGGCTGGA CCATTGGCGT ATGCTCGTCT GCCACTTGGT CCTCTAATTG AAAGTTTTTT CGAAATTGTT

11101 CCACGTGCGT ACGCTTGTGG CGCGCGAGGA GGTGGCTATA GGACTGATGC ATCTGTGGGA CTTTGTAAGC GCGCTGGAGC AAAACCCAAA TAGCAAGCCG
GGTGCACGCA TGCGAACACC GCGCGCTCCT CCACCGATAT CCTGACTACG TAGACACCCT GAAACATTCG CGCGACCTCG TTTTGGGTTT ATCGTTCGGC

11201 CTCATGGCGC AGCTGTTCCT TATAGTGCAG CACAGCAGGG ACAACGAGGC ATTCAGGGAT GCGCTGCTAA ACATAGTAGA GCCCGAGGGC CGCTGGCTGC
GAGTACCGCG TCGACAAGGA ATATCACGTC GTGTCGTCCC TGTTGCTCCG TAAGTCCCTA CGCGACGATT TGTATCATCT CGGGCTCCCG GCGACCGACG

Figure 15G

pMRKAd5gag MRKB2

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                              PstI
11301  TCGATTTGAT AAACATCCTG CAGAGCATAG TGGTGCAGGA GCGCAGCTTG AGCCTGGCTG ACAAGGTGGC CGCCATCAAC TATTCCATGC TTAGCCTGGG
       AGCTAAACTA TTTGTAGGAC GTCTCGTATC ACCACGTCCT CGCGTCGAAC TCGGACCGAC TGTTCCACCG GCGGTAGTTG ATAAGGTACG AATCGGACCC
11401  CAAGTTTTAC GCCCGCAAGA TATACCATAC CCCTTACGTT CCCATAGACA AGGAGGTAAA GATCGAGGGG TTCTACATGC GCATGCGCCT GAAGGTGCTG
       GTTCAAAATG CGGGCGTTCT ATATGGTATG GGGAATGCAA GGGTATCTGT TCCTCCATTT CTAGCTCCCC AAGATGTACG CGTACGCGGA CTTCCACGAC
11501  ACCTTGAGCG ACGACCTGGG CGTTTATCGC AACGAGCGCA TCCACAAGGC CGTGAGCGTG AGCCCGCGGC GCGAGCTCAG CGACCGCGAG CTGATGCACA
       TGGAACTCGC TGCTGGACCC GCAAATAGCG TTGCTCGCGT AGGTGTTCCG GCACTCGCAC TCGGGCGCCG CGCTCGAGTC GCTGGCGCTC GACTACGTGT
11601  GCCTGCAAAG GGCCCTGGCT GGCACCGGCA GCGGCGATAG AGAGGCCGAG TCCTACTTTG ACGCGGGCGC TGACCTGCGC TGGGCCCCAA GCCGACGCGC
       CGGACGTTTC CCGGGACCGA CCGTGGCCGT CGCCGCTATC TCTCCGGCTC AGGATGAAAC TGCGCCCGCG ACTGGACGCG ACCCGGGGTT CGGCTGCGCG
11701  CCTGGAGGCA GCTGGGGCCG GACCTGGGCT GGCGGTGGCA CCCCGCGGCG CTGGCAACGT CGGCGGCGTG GAGGAATATG ACGAGGACGA TGAGTACGAG
       GGACCTCCGT CGACCCCGGC CTGGACCCGA CCGCCACCGT GGGGCGCCGC GACCGTTGCA GCCGCCGCAC CTCCTTATAC TGCTCCTGCT ACTCATGCTC
                                                                                                  PstI
11801  CCAGAGGACG GCGAGTACTA AGCGGTGATG TTTCTGATCA GATGATGCAA GACGCAACGG ACCCGGCGGT GCGGGCGGCG CTGCAGAGCC AGCCGTCCGG
       GGTCTCCTGC CGCTCATGAT TCGCCACTAC AAAGACTAGT CTACTACGTT CTGCGTTGCC TGGGCCGCCA CGCCCGCCGC GACGTCTCGG TCGGCAGGCC
11901  CCTTAACTCC ACGGACGACT GGCGCCAGGT CATGGACCGC ATCATGTCGC TGACTGCGCG CAATCCTGAC GCGTTCCGGC AGCAGCCGCA GGCCAACCGG
       GGAATTGAGG TGCCTGCTGA CCGCGGTCCA GTACCTGGCG TAGTACAGCG ACTGACGCGC GTTAGGACTG CGCAAGGCCG TCGTCGGCGT CCGGTTGGCC
                                                                              PvuI
12001  CTCTCCGCAA TTCTGGAAGC GGTGGTCCCG GCGCGCGCAA ACCCCACGCA CGAGAAGGTG CTGGCGATCG TAAACGCGCT GGCCGAAAAC AGGGCCATCC
       GAGAGGCGTT AAGACCTTCG CCACCAGGGC CGCGCGCGTT TGGGGTGCGT GCTCTTCCAC GACCGCTAGC ATTTGCGCGA CCGGCTTTTG TCCCGGTAGG
12101  GGCCCGACGA GGCCGGCCTG GTCTACGACG CGCTGCTTCA GCGCGTGGCT CGTTACAACA GCGGCAACGT GCAGACCAAC CTGGACCGGC TGGTGGGGGA
       CCGGGCTGCT CCGGCCGGAC CAGATGCTGC GCGACGAAGT CGCGCACCGA GCAATGTTGT CGCCGTTGCA CGTCTGGTTG GACCTGGCCG ACCACCCCCT
12201  TGTGCGCGAG GCCGTGGCGC AGCGTGAGCG CGCGCAGCAG CAGGGCAACC TGGGCTCCAT GGTTGCACTA AACGCCTTCC TGAGTACACA GCCCGCCAAC
       ACACGCGCTC CGGCACCGCG TCGCACTCGC GCGCGTCGTC GTCCCGTTGG ACCCGAGGTA CCAACGTGAT TTGCGGAAGG ACTCATGTGT CGGGCGGTTG
12301  GTGCCGCGGG GACAGGAGGA CTACACCAAC TTTGTGAGCG CACTGCGGCT AATGGTGACT GAGACACCGC AAAGTGAGGT GTACCAGTCT GGGCCAGACT
       CACGGCGCCC CTGTCCTCCT GATGTGGTTG AAACACTCGC GTGACGCCGA TTACCACTGA CTCTGTGGCG TTTCACTCCA CATGGTCAGA CCCGGTCTGA
                                    PstI
12401  ATTTTTTCCA GACCAGTAGA CAAGGCCTGC AGACCGTAAA CCTGAGCCAG GCTTTCAAAA ACTTGCAGGG GCTGTGGGGG GTGCGGGCTC CCACAGGCGA
       TAAAAAAGGT CTGGTCATCT GTTCCGGACG TCTGGCATTT GGACTCGGTC CGAAAGTTTT TGAACGTCCC CGACACCCCC CACGCCCGAG GGTGTCCGCT
12501  CCGCGCGACC GTGTCTAGCT TGCTGACGCC CAACTCGCGC CTGTTGCTGC TGCTAATAGC GCCCTTCACG GACAGTGGCA GCGTGTCCCG GGACACATAC
       GGCGCGCTGG CACAGATCGA ACGACTGCGG GTTGAGCGCG GACAACGACG ACGATTATCG CGGGAAGTGC CTGTCACCGT CGCACAGGGC CCTGTGTATG
12601  CTAGGTCACT TGCTGACACT GTACCGCGAG GCCATAGGTC AGGCGCATGT GGACGAGCAT ACTTTCCAGG AGATTACAAG TGTCAGCCGC GCGCTGGGGC
       GATCCAGTGA ACGACTGTGA CATGGCGCTC CGGTATCCAG TCCGCGTACA CCTGCTCGTA TGAAAGGTCC TCTAATGTTC ACAGTCGGCG CGCGACCCCG
                                                                                               PmeI
12701  AGGAGGACAC GGGCAGCCTG GAGGCAACCC TAAACTACCT GCTGACCAAC CGGCGGCAGA AGATCCCCTC GTTGCACAGT TTAAACAGCG AGGAGGAGCG
       TCCTCCTGTG CCCGTCGGAC CTCCGTTGGG ATTTGATGGA CGACTGGTTG GCCGCCGTCT TCTAGGGGAG CAACGTGTCA AATTTGTCGC TCCTCCTCGC
12801  CATTTTGCGC TACGTGCAGC AGAGCGTGAG CCTTAACCTG ATGCGCGACG GGGTAACGCC CAGCGTGGCG CTGGACATGA CCGCGCGCAA CATGGAACCG
       GTAAAACGCG ATGCACGTCG TCTCGCACTC GGAATTGGAC TACGCGCTGC CCCATTGCGG GTCGCACCGC GACCTGTACT GGCGCGCGTT GTACCTTGGC
```

Figure 15H

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12901  GGCATGTATG CCTCAAACCG GCCGTTTATC AACCGCCTAA TGGACTACTT GCATCGCGTG GCCGCCGTGA ACCCCGAGTA TTTCACCAAT GCCATCTTGA
       CCGTACATAC GGAGTTTGGC CGGCAAATAG TTGGCGGATT ACCTGATGAA CGTAGCGCAC CGGCGGCACT TGGGGCTCAT AAAGTGGTTA CGGTAGAACT
13001  ACCCGCACTG GCTACCGCCC CCTGGTTTCT ACACCGGGGG ATTCGAGGTG CCCGAGGGTA ACGATGGATT CCTCTGGGAC GACATAGACG ACAGCGTGTT
       TGGGCGTGAC CGATGGCGGG GGACCAAAGA TGTGGCCCCC TAAGCTCCAC GGGCTCCCAT TGCTACCTAA GGAGACCCTG CTGTATCTGC TGTCGCACAA
                                                                                  HindIII
13101  TTCCCCGCAA CCGCAGACCC TGCTAGAGTT GCAACAGGGC GAGCAGGCAG AGGCGGCGGT GCGAAAGGAA AGCTTCCGCA GGCCAAGCAG CTTGTCCGAT
       AAGGGGCGTT GGCGTCTGGG ACGATCTCAA CGTTGTCCCG CTCGTCCGTC TCCGCCGCCA CGCTTTCCTT TCGAAGGCGT CCGGTTCGTC GAACAGGCTA
                                                    HindIII
13201  CTAGGCGCTG CGGCCCCGCG GTCAGATGCT AGTAGCCCAT TTCCAAGCTT GATAGGGTCT CTTACCAGCA CTCGCACCAC CCGCCGCGGC CTGCTGGGG
       GATCCGCGAC GCCGGGGCGC CAGTCTACGA TCATCGGGTA AAGGTTCGAA CTATCCCAGA GAATGGTCGT GAGCGTGGTG GGCGGCGCCG GACGACCCC
                         PstI
13301  AGGAGGAGTA CCTAAACAAC TCGCTGCAGC AGCCGCAGCG CGAAAAAAAC CTGCCTCCGG CATTTCCCAA CAACGGGATA GAGAGCCTAG TGGACAAGAT
       TCCTCCTCAT GGATTTGTTG AGCGACGTCG TCGGCGTCGC GCTTTTTTTG GACGGAGGCC GTAAAGGGTT GTTGCCCTAT CTCTCGGATC ACCTGTTCTA
13401  GAGTAGATGG AAGACGTACG CGCAGGAGCA CAGGGACGTG CCAGGCCCGC GCCCGCCCAC CCGTCGTCAA AGGCACGACC GTCAGCGGGG TCTGGTGTGG
       CTCATCTACC TTCTGCATGC GCGTCCTCGT GTCCCTGCAC GGTCCGGGCG CGGGCGGGTG GGCAGCAGTT TCCGTGCTGG CAGTCGCCCC AGACCACACC
13501  GAGGACGATG ACTCGGCAGA CGACAGCAGC GTCCTGGATT TGGGAGGGAG TGGCAACCCG TTTGCGCACC TTCGCCCCAG GCTGGGGAGA ATGTTTTAAA
       CTCCTGCTAC TGAGCCGTCT GCTGTCGTCG CAGGACCTAA ACCCTCCCTC ACCGTTGGGC AAACGCGTGG AAGCGGGGTC CGACCCCTCT TACAAAATTT
13601  AAAAAAAAAA GCATGATGCA AAATAAAAAA CTCACCAAGG CCATGGCACC GAGCGTTGGT TTTCTTGTAT TCCCCTTAGT ATGCGGCGCG CGGCGATGTA
       TTTTTTTTTT CGTACTACGT TTTATTTTTT GAGTGGTTCC GGTACCGTGG CTCGCAACCA AAAGAACATA AGGGGAATCA TACGCCGCGC GCCGCTACAT
13701  TGAGGAAGGT CCTCCTCCCT CCTACGAGAG TGTGGTGAGC GCGGGCGCAG TGGCGGCGGC GCTGGGTTCT CCCTTCGATG CTCCCCTGGA CCCGCCGTTT
       ACTCCTTCCA GGAGGAGGGA GGATGCTCTC ACACCACTCG CGCCCGCGTC ACCGCCGCCG CGACCCAAGA GGGAAGCTAC GAGGGGACCT GGGCGGCAA..
                  KpnI
13801  GTGCCTCCGC GGTACCTGCG GGCTACCGGG GGGAGAAACA GCATCCGTTA CTCTGAGTTG GCACCCCTAT TCGACACCAC CCGTGTGTAC CTGGTGGACA
       CACGGAGGCG CCATGGACGC CCGATGGCCC CCCTCTTTGT CGTAGGCAAT GAGACTCAAC CGTGGGGATA AGCTGTGGTG GGCACACATG GACCACCTGT
13901  ACAAGTCAAC GGATGTGGCA TCCCTGAACT ACCAGAACGA CCACAGCAAC TTTCTGACCA CGGTCATTCA AAACAATGAC TACAGCCCGG GGGAGGCAAG
       TGTTCAGTTG CCTACACCGT AGGGACTTGA TGGTCTTGCT GGTGTCGTTG AAAGACTGGT GCCAGTAAGT TTTGTTACTG ATGTCGGGCC CCCTCCGTTC
14001  CACACAGACC ATCAATCTTG ACGACCGGTC GCACTGGGGC GGCGACCTGA AAACCATCCT GCATACCAAC ATGCCAAATG TGAACGAGTT CATGTTTACC
       GTGTGTCTGG TAGTTAGAAC TGCTGGCCAG CGTGACCCCG CCGCTGGACT TTTGGTAGGA CGTATGGTTG TACGGTTTAC ACTTGCTCAA GTACAAATGG
14101  AATAAGTTTA AGGCGCGGGT GATGGTGTCG CGCTTGCCTA CTAAGGACAA TCAGGTGGAG CTGAAATACG AGTGGGTGGA GTTCACGCTG CCCGAGGGCA
       TTATTCAAAT TCCGCGCCCA CTACCACAGC GCGAACGGAT GATTCCTGTT AGTCCACCTC GACTTTATGC TCACCCACCT CAAGTGCGAC GGGCTCCCGT
                                                PvuI
14201  ACTACTCCGA GACCATGACC ATAGACCTTA TGAACAACGC GATCGTGGAG CACTACTTGA AAGTGGGCAG ACAGAACGGG GTTCTGGAAA GCGACATCGG
       TGATGAGGCT CTGGTACTGG TATCTGGAAT ACTTGTTGCG CTAGCACCTC GTGATGAACT TTCACCCGTC TGTCTTGCCC CAAGACCTTT CGCTGTAGCC
14301  GGTAAAGTTT GACACCCGCA ACTTCAGACT GGGGTTTGAC CCCGTCACTG GTCTTGTCAT GCCTGGGGTA TATACAAACG AAGCCTTCCA TCCAGACATC
       CCATTTCAAA CTGTGGGCGT TGAAGTCTGA CCCCAAACTG GGGCAGTGAC CAGAACAGTA CGGACCCCAT ATATGTTTGC TTCGGAAGGT AGGTCTGTAG
14401  ATTTTGCTGC CAGGATGCGG GGTGGACTTC ACCCACAGCC GCCTGAGCAA CTTGTTGGGC ATCCGCAAGC GGCAACCCTT CCAGGAGGGC TTTAGGATCA
       TAAAACGACG GTCCTACGCC CCACCTGAAG TGGGTGTCGG CGGACTCGTT GAACAACCCG TAGGCGTTCG CCGTTGGGAA GGTCCTCCCG AAATCCTAGT
```

Figure 15I

pMRKAd5gag HER6R2

14501 CCTACGATGA TCTGGAGGGT GGTAACATTC CCGCACTGTT GGATGTGGAC GCCTACCAGG CGAGCTTGAA AGATGACACC GAACAGGGCG GGGGTGGCGC
GGATGCTACT AGACCTCCCA CCATTGTAAG GGCGTGACAA CCTACACCTG CGGATGGTCC GCTCGAACTT TCTACTGTGG CTTGTCCCGC CCCCACCGCG

14601 AGGCGGCAGC AACAGCAGTG GCAGCGGCGC GGAAGAGAAC TCCAACGCGG CAGCCGCGGC AATGCAGCCG GTGGAGGACA TGAACGATCA TGCCATTCGC
TCCGCCGTCG TTGTCGTCAC CGTCGCCGCG CCTTCTCTTG AGGTTGCGCC GTCGGCGCCG TTACGTCGGC CACCTCCTGT ACTTGCTAGT ACGGTAAGCG

14701 GGCGACACCT TTGCCACACG GGCTGAGGAG AAGCGCGCTG AGGCCGAAGC AGCGGCCGAA GCTGCCGCTC CCGCTGCGCA ACCCGAGGTC GAGAAGCCTC
CCGCTGTGGA AACGGTGTGC CCGACTCCTC TTCGCGCGAC TCCGGCTTCG TCGCCGGCTT CGACGGCGAG GGCGACGCGT TGGGCTCCAG CTCTTCGGAG
KpnI

14801 AGAAGAAACC GGTGATCAAA CCCCTGACAG AGGACAGCAA GAAACGCAGT TACAACCTAA TAAGCAATGA CAGCACCTTC ACCCAGTACC GCAGCTGGTA
TCTTCTTTGG CCACTAGTTT GGGGACTGTC TCCTGTCGTT CTTTGCGTCA ATGTTGGATT ATTCGTTACT GTCGTGGAAG TGGGTCATGG CGTCGACCAT

Kpn

14901 CCTTGCATAC AACTACGGCG ACCCTCAGAC CGGAATCCGC TCATGGACCC TGCTTTGCAC TCCTGACGTA ACCTGCGGCT CGGAGCAGGT CTACTGGTCG
GGAACGTATG TTGATGCCGC TGGGAGTCTG GCCTTAGGCG AGTACCTGGG ACGAAACGTG AGGACTGCAT TGGACGCCGA GCCTCGTCCA GATGACCAGC

15001 TTGCCAGACA TGATGCAAGA CCCCGTGACC TTCCGCTCCA CGCGCCAGAT CAGCAACTTT CCGGTGGTGG GCGCCGAGCT GTTGCCCGTG CACTCCAAGA
AACGGTCTGT ACTACGTTCT GGGGCACTGG AAGGCGAGGT GCGCGGTCTA GTCGTTGAAA GGCCACCACC CGCGGCTCGA CAACGGGCAC GTGAGGTTCT
AscI

15101 GCTTCTACAA CGACCAGGCC GTCTACTCCC AACTCATCCG CCAGTTTACC TCTCTGACCC ACGTGTTCAA TCGCTTTCCC GAGAACCAGA TTTTGGCGCG
CGAAGATGTT GCTGGTCCGG CAGATGAGGG TTGAGTAGGC GGTCAAATGG AGAGACTGGG TGCACAAGTT AGCGAAAGGG CTCTTGGTCT AAAACCGCGC

AscI

15201 CCCGCCAGCC CCCACCATCA CCACCGTCAG TGAAAACGTT CCTGCTCTCA CAGATCACGG GACGCTACCG CTGCGCAACA GCATCGGAGG AGTCCAGCGA
GGGCGGTCGG GGGTGGTAGT GGTGGCAGTC ACTTTTGCAA GGACGAGAGT GTCTAGTGCC CTGCGATGGC GACGCGTTGT CGTAGCCTCC TCAGGTCGCT

15301 GTGACCATTA CTGACGCCAG ACGCCGCACC TGCCCCTACG TTTACAAGGC CCTGGGCATA GTCTCGCCGC GCGTCCTATC GAGCCGCACT TTTTGAGCAA
CACTGGTAAT GACTGCGGTC TGCGGCGTGG ACGGGGATGC AAATGTTCCG GGACCCGTAT CAGAGCGGCG CGCAGGATAG CTCGGCGTGA AAAACTCGTT

15401 GCATGTCCAT CCTTATATCG CCCAGCAATA ACACAGGCTG GGGCCTGCGC TTCCCAAGCA AGATGTTTGG CGGGGCCAAG AAGCGCTCCG ACCAACACCC
CGTACAGGTA GGAATATAGC GGGTCGTTAT TGTGTCCGAC CCCGGACGCG AAGGGTTCGT TCTACAAACC GCCCCGGTTC TTCGCGAGGC TGGTTGTGGG

15501 AGTGCGCGTG CGCGGGCACT ACCGCGCGCC CTGGGGCGCG CACAAACGCG GCCGCACTGG GCGCACCACC GTCGATGACG CCATCGACGC GGTGGTGGAG
TCACGCGCAC GCGCCCGTGA TGGCGCGCGG GACCCCGCGC GTGTTTGCGC CGGCGTGACC CGCGTGGTGG CAGCTACTGC GGTAGCTGCG CCACCACCTC

15601 GAGGCGCGCA ACTACACGCC CACGCCGCCA CCAGTGTCCA CAGTGGACGC GGCCATTCAG ACCGTGGTGC GCGGAGCCCG GCGCTATGCT AAAATGAAGA
CTCCGCGCGT TGATGTGCGG GTGCGGCGGT GGTCACAGGT GTCACCTGCG CCGGTAAGTC TGGCACCACG CGCCTCGGGC CGCGATACGA TTTTACTTCT

15701 GACGGCGGAG GCGCGTAGCA CGTCGCCACC GCCGCCGACC CGGCACTGCC GCCCAACGCG CGGCGGCGGC CCTGCTTAAC CGCGCACGTC GCACCGGCCG
CTGCCGCCTC CGCGCATCGT GCAGCGGTGG CGGCGGCTGG GCCGTGACGG CGGGTTGCGC GCCGCCGCCG GGACGAATTG GCGCGTGCAG CGTGGCCGGC

SfiI

15801 ACGGGCGGCC ATGCGGGCCG CTCGAAGGCT GGCCGCGGGT ATTGTCACTG TGCCCCCCAG GTCCAGGCGA CGAGCGGCCG CCGCAGCAGC CGCGGCCATT
TGCCCGCCGG TACGCCCGGC GAGCTTCCGA CCGGCGCCCA TAACAGTGAC ACGGGGGGTC CAGGTCCGCT GCTCGCCGGC GGCGTCGTCG GCGCCGGTAA

15901 AGTGCTATGA CTCAGGGTCG CAGGGGCAAC GTGTATTGGG TGCGCGACTC GGTTAGCGGC CTGCGCGTGC CCGTGCGCAC CCGCCCCCCG CGCAACTAGA
TCACGATACT GAGTCCCAGC GTCCCCGTTG CACATAACCC ACGCGCTGAG CCAATCGCCG GACGCGCACG GGCACGCGTG GGCGGGGGGC GCGTTGATCT

16001 TTGCAAGAAA AAACTACTTA GACTCGTACT GTTGTATGTA TCCAGCGGCG GCGGCGCGCA ACGAAGCTAT GTCCAAGCGC AAAATCAAAG AAGAGATGCT
AACGTTCTTT TTTGATGAAT CTGAGCATGA CAACATACAT AGGTCGCCGC CGCCGCGCGT TGCTTCGATA CAGGTTCGCG TTTTAGTTTC TTCTCTACGA

Figure 15J

pMRKAd5gag MER682

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                      BglII
16101  CCAGGTCATC GCGCCGGAGA TCTATGGCCC CCCGAAGAAG GAAGAGCAGG ATTACAAGCC CCGAAAGCTA AAGCGGGTCA AAAAGAAAAA GAAAGATGAT
       GGTCCAGTAG CGCGGCCTCT AGATACCGGG GGGCTTCTTC CTTCTCGTCC TAATGTTCGG GGCTTTCGAT TTCGCCCAGT TTTTCTTTTT CTTTCTACTA
                                                                                  SalI
16201  GATGATGAAC TTGACGACGA GGTGGAACTG CTGCACGCTA CCGCGCCCAG GCGACGGGTA CAGTGGAAAG GTCGACGCGT AAAACGTGTT TTGCGACCCG
       CTACTACTTG AACTGCTGCT CCACCTTGAC GACGTGCGAT GGCGCGGGTC CGCTGCCCAT GTCACCTTTC CAGCTGCGCA TTTTGCACAA AACGCTGGGC
16301  GCACCACCGT AGTCTTTACG CCCGGTGAGC GCTCCACCCG CACCTACAAG CGCGTGTATG ATGAGGTGTA CGGCGACGAG GACCTGCTTG AGCAGGCCAA
       CGTGGTGGCA TCAGAAATGC GGGCCACTCG CGAGGTGGGC GTGGATGTTC GCGCACATAC TACTCCACAT GCCGCTGCTC CTGGACGAAC TCGTCCGGTT
16401  CGAGCGCCTC GGGGAGTTTG CCTACGGAAA GCGGCATAAG GACATGCTGG CGTTGCCGCT GGACGAGGGC AACCCAACAC CTAGCCTAAA GCCCGTAACA
       GCTCGCGGAG CCCCTCAAAC GGATGCCTTT CGCCGTATTC CTGTACGACC GCAACGGCGA CCTGCTCCCG TTGGGTTGTG GATCGGATTT CGGGCATTGT
        PstI                                                                                                   KpnI
16501  CTGCAGCAGG TGCTGCCCGC GCTTGCACCG TCCGAAGAAA AGCGCGGCCT AAAGCGCGAG TCTGGTGACT TGGCACCCAC CGTGCAGCTG ATGGTACCCA
       GACGTCGTCC ACGACGGGCG CGAACGTGGC AGGCTTCTTT TCGCGCCGGA TTTCGCGCTC AGACCACTGA ACCGTGGGTG GCACGTCGAC TACCATGGGT
16601  AGCGCCAGCG ACTGGAAGAT GTCTTGGAAA AAATGACCGT GGAACCTGGG CTGGAGCCCG AGGTCCGCGT GCGGCCAATC AAGCAGGTGG CGCCGGGACT
       TCGCGGTCGC TGACCTTCTA CAGAACCTTT TTTACTGGCA CCTTGGACCC GACCTCGGGC TCCAGGCGCA CGCCGGTTAG TTCGTCCACC GCGGCCCTGA
16701  GGGCGTGCAG ACCGTGGACG TTCAGATACC CACTACCAGT AGCACCAGTA TTGCCACCGC CACAGAGGGC ATGGAGACAC AAACGTCCCC GGTTGCCTCA
       CCCGCACGTC TGGCACCTGC AAGTCTATGG GTGATGGTCA TCGTGGTCAT AACGGTGGCG GTGTCTCCCG TACCTCTGTG TTTGCAGGGG CCAACGGAGT
16801  GCGGTGGCGG ATGCCGCGGT GCAGGCGGTC GCTGCGGCCG CGTCCAAGAC CTCTACGGAG GTGCAAACGG ACCCGTGGAT GTTTCGCGTT TCAGCCCCCC
       CGCCACCGCC TACGGCGCCA CGTCCGCCAG CGACGCCGGC GCAGGTTCTG GAGATGCCTC CACGTTTGCC TGGGCACCTA CAAAGCGCAA AGTCGGGGGG
16901  GGCGCCCGCG CCGTTCGAGG AAGTACGGCG CCGCCAGCGC GCTACTGCCC GAATATGCCC TACATCCTTC CATTGCGCCT ACCCCCGGCT ATCGTGGCT
       CCGCGGGCGC GGCAAGCTCC TTCATGCCGC GGCGGTCGCG CGATGACGGG CTTATACGGG ATGTAGGAAG GTAACGCGGA TGGGGGCCGA TAGCACCGAT
17001  CACCTACCGC CCCAGAAGAC GAGCAACTAC CCGACGCCGA ACCACCACTG GAACCCGCCG CCGCCGTCGC CGTCGCCAGC CCGTGCTGGC CCCGATTTCC
       GTGGATGGCG GGGTCTTCTG CTCGTTGATG GGCTGCGGCT TGGTGGTGAC CTTGGGCGGC GGCGGCAGCG GCAGCGGTCG GGCACGACCG GGGCTAAAGG
17101  GTGCGCAGGG TGGCTCGCGA AGGAGGCAGG ACCCTGGTGC TGCCAACAGC GCGCTACCAC CCCAGCATCG TTTAAAAGCC GGTCTTTGTG GTTCTTGCAG
       CACGCGTCCC ACCGAGCGCT TCCTCCGTCC TGGGACCACG ACGGTTGTCG CGCGATGGTG GGGTCGTAGC AAATTTTCGG CCAGAAACAC CAAGAACGTC
                                                                                                             SphI
17201  ATATGGCCCT CACCTGCCGC CTCCGTTTCC CGGTGCCGGG ATTCCGAGGA AGAATGCACC GTAGGAGGGG CATGGCCGGC CACGGCCTGA CGGGCGGCAT
       TATACCGGGA GTGGACGGCG GAGGCAAAGG GCCACGGCCC TAAGGCTCCT TCTTACGTGG CATCCTCCCC GTACCGGCCG GTGCCGGACT GCCCGCCGTA
       Sph                                         SphI
17301  GCGTCGTGCG CACCACCGGC GGCGGCGCGC GTCGCACCGT CGCATGCGCG GCGGTATCCT GCCCCTCCTT ATTCCACTGA TCGCCGCGGC GATTGGCGCC
       CGCAGCACGC GTGGTGGCCG CCGCCGCGCG CAGCGTGGCA GCGTACGCGC CGCCATAGGA CGGGGAGGAA TAAGGTGACT AGCGGCGCCG CTAACCGCGG
17401  GTGCCCGGAA TTGCATCCGT GGCCTTGCAG GCGCAGAGAC ACTGATTAAA AACAAGTTGC ATGTGGAAAA ATCAAAATAA AAAGTCTGGA CTCTCACGCT
       CACGGGCCTT AACGTAGGCA CCGGAACGTC CGCGTCTCTG TGACTAATTT TTGTTCAACG TACACCTTTT TAGTTTTATT TTTCAGACCT GAGAGTGCGA
                                                                                                            EcoRV
17501  CGCTTGGTCC TGTAACTATT TTGTAGAATG GAAGACATCA ACTTTGCGTC TCTGGCCCCG CGACACGGCT CGCGCCCGTT CATGGGAAAC TGGCAAGATA
       GCGAACCAGG ACATTGATAA AACATCTTAC CTTCTGTAGT TGAAACGCAG AGACCGGGGC GCTGTGCCGA GCGCGGGCAA GTACCCTTTG ACCGTTCTAT
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Figure 15K

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      EcoRV
17601 TCGGCACCAG CAATATGAGC GGTGGCGCCT TCAGCTGGGG CTCGCTGTGG AGCGGCATTA AAAATTTCGG TTCCACCGTT AAGAACTATG GCAGCAAGG
      AGCCGTGGTC GTTATACTCG CCACCGCGGA AGTCGACCCC GAGCGACACC TCGCCGTAAT TTTTAAAGCC AAGGTGGCAA TTCTTGATAC CGTCGTTCC
17701 CTGGAACAGC AGCACAGGCC AGATGCTGAG GGATAAGTTG AAAGAGCAAA ATTTCCAACA AAAGGTGGTA GATGGCCTGG CCTCTGGCAT TAGCGGGGT
      GACCTTGTCG TCGTGTCCGG TCTACGACTC CCTATTCAAC TTTCTCGTTT TAAAGGTTGT TTTCCACCAT CTACCGGACC GGAGACCGTA ATCGCCCCA
                                                HindIII
17801 GTGGACCTGG CCAACCAGGC AGTGCAAAAT AAGATTAACA GTAAGCTTGA TCCCCGCCCT CCCGTAGAGG AGCCTCCACC GGCCGTGGAG ACAGTGTCT
      CACCTGGACC GGTTGGTCCG TCACGTTTTA TTCTAATTGT CATTCGAACT AGGGGCGGGA GGGCATCTCC TCGGAGGTGG CCGGCACCTC TGTCACAG
17901 CAGAGGGGCG TGGCGAAAAG CGTCCGCGCC CCGACAGGGA AGAAACTCTG GTGACGCAAA TAGACGAGCC TCCCTCGTAC GAGGAGGCAC TAAAGCAA
      GTCTCCCCGC ACCGCTTTTC GCAGGCGCGG GGCTGTCCCT TCTTTGAGAC CACTGCGTTT ATCTGCTCGG AGGGAGCATG CTCCTCCGTG ATTTCGTT
18001 CCTGCCCACC ACCCGTCCCA TCGCGCCCAT GGCTACCGGA GTGCTGGGCC AGCACACACC CGTAACGCTG GACCTGCCTC CCCCCGCCGA CACCCAGCA
      GGACGGGTGG TGGGCAGGGT AGCGCGGGTA CCGATGGCCT CACGACCCGG TCGTGTGTGG GCATTGCGAC CTGGACGGAG GGGGGCGGCT GTGGGTCGT
                                                                                                PvuI
18101 AAACCTGTGC TGCCAGGCCC GACCGCCGTT GTTGTAACCC GTCCTAGCCG CGCGTCCCTG CGCCGCGCCG CCAGCGGTCC GCGATCGTTG CGGCCCGTAG
      TTTGGACACG ACGGTCCGGG CTGGCGGCAA CAACATTGGG CAGGATCGGC GCGCAGGGAC GCGGCGCGGC GGTCGCCAGG CGCTAGCAAC GCCGGGCATC
18201 CCAGTGGCAA CTGGCAAAGC ACACTGAACA GCATCGTGGG TCTGGGGGTG CAATCCCTGA AGCGCCGACG ATGCTTCTGA TAGCTAACGT GTCGTATGT
      GGTCACCGTT GACCGTTTCG TGTGACTTGT CGTAGCACCC AGACCCCCAC GTTAGGGACT TCGCGGCTGC TACGAAGACT ATCGATTGCA CAGCATACA
18301 TGTCATGTAT GCGTCCATGT CGCCGCCAGA GGAGCTGCTG AGCCGCCGCG CGCCCGCTTT CCAAGATGGC TACCCCTTCG ATGATGCCGC AGTGGTCTTA
      ACAGTACATA CGCAGGTACA GCGGCGGTCT CCTCGACGAC TCGGCGGCGC GCGGGCGAAA GGTTCTACCG ATGGGGAAGC TACTACGGCG TCACCAGA
18401 CATGCACATC TCGGGCCAGG ACGCCTCGGA GTACCTGAGC CCCGGGCTGG TGCAGTTTGC CCGCGCCACC GAGACGTACT TCAGCCTGAA TAACAAGTTT
      GTACGTGTAG AGCCCGGTCC TGCGGAGCCT CATGGACTCG GGGCCCGACC ACGTCAAACG GGCGCGGTGG CTCTGCATGA AGTCGGACTT ATTGTTCAAA
18501 AGAAACCCCA CGGTGGCGCC TACGCACGAC GTGACCACAG ACCGGTCCCA GCGTTTGACG CTGCGGTTCA TCCCTGTGGA CCGTGAGGAT ACTGCGTACT
      TCTTTGGGGT GCCACCGCGG ATGCGTGCTG CACTGGTGTC TGGCCAGGGT CGCAAACTGC GACGCCAAGT AGGGACACCT GGCACTCCTA TGACGCATGA
18601 CGTACAAGGC GCGGTTCACC CTAGCTGTGG GTGATAACCG TGTGCTGGAC ATGGCTTCCA CGTACTTTGA CATCCGCGGC GTGCTGGACA GGGGCCCTA
      GCATGTTCCG CGCCAAGTGG GATCGACACC CACTATTGGC ACACGACCTG TACCGAAGGT GCATGAAACT GTAGGCGCCG CACGACCTGT CCCCGGGAT
18701 TTTTAAGCCC TACTCTGGCA CTGCCTACAA CGCCCTGGCT CCCAAGGGTG CCCCAAATCC TTGCGAATGG GATGAAGCTG CTACTGCTCT TGAAATAAA
      AAAATTCGGG ATGAGACCGT GACGGATGTT GCGGGACCGA GGGTTCCCAC GGGGTTTAGG AACGCTTACC CTACTTCGAC GATGACGAGA ACTTTATTT
18801 CTAGAAGAAG AGGACGATGA CAACGAAGAC GAAGTAGACG AGCAAGCTGA GCAGCAAAAA ACTCACGTAT TTGGGCAGGC GCCTTATTCT GGTATAAATA
      GATCTTCTTC TCCTGCTACT GTTGCTTCTG CTTCATCTGC TCGTTCGACT CGTCGTTTTT TGAGTGCATA AACCCGTCCG CGGAATAAGA CCATATTTAT
18901 TTACAAAGGA GGGTATTCAA ATAGGTGTCG AAGGTCAAAC ACCTAAATAT GCCGATAAAA CATTTCAACC TGAACCTCAA ATAGGAGAAT CTCAGTGGTA
      AATGTTTCCT CCCATAAGTT TATCCACAGC TTCCAGTTTG TGGATTTATA CGGCTATTTT GTAAAGTTGG ACTTGGAGTT TATCCTCTTA GAGTCACCAT
19001 CGAAACAGAA ATTAATCATG CAGCTGGGAG AGTCCTAAAA AAGACTACCC CAATGAAACC ATGTTACGGT TCATATGCAA AACCCACAAA TGAAAATGG
      GCTTTGTCTT TAATTAGTAC GTCGACCCTC TCAGGATTTT TTCTGATGGG GTTACTTTGG TACAATGCCA AGTATACGTT TTGGGTGTTT ACTTTTACC
19101 GGGCAAGGCA TTCTTGTAAA GCAACAAAAT GGAAAGCTAG AAAGTCAAGT GGAAATGCAA TTTTTCTCAA CTACTGAGGC AGCCGCAGGC AATGGTGAT
      CCCGTTCCGT AAGAACATTT CGTTGTTTTA CCTTTCGATC TTTCAGTTCA CCTTTACGTT AAAAAGAGTT GATGACTCCG TCGGCGTCCG TTACCACTA
19201 ACTTGACTCC TAAAGTGGTA TTGTACAGTG AAGATGTAGA TATAGAAACC CCAGACACTC ATATTTCTTA CATGCCCACT ATTAAGGAAG GTAACTCAC
      TGAACTGAGG ATTTCACCAT AACATGTCAC TTCTACATCT ATATCTTTGG GGTCTGTGAG TATAAAGAAT GTACGGGTGA TAATTCCTTC CATTGAGTG
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Figure 15L

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19301  AGAACTAATG GGCCAACAAT CTATGCCCAA CAGGCCTAAT TACATTGCTT TTAGGGACAA TTTTATTGGT CTAATGTATT ACAACAGCAC GGGTAATATG
       TCTTGATTAC CCGGTTGTTA GATACGGGTT GTCCGGATTA ATGTAACGAA AATCCCTGTT AAAATAACCA GATTACATAA TGTTGTCGTG CCCATTATAC
19401  GGTGTTCTGG CGGGCCAAGC ATCGCAGTTG AATGCTGTTG TAGATTTGCA AGACAGAAAC ACAGAGCTTT CATACCAGCT TTTGCTTGAT TCCATTGGTG
       CCACAAGACC GCCCGGTTCG TAGCGTCAAC TTACGACAAC ATCTAAACGT TCTGTCTTTG TGTCTCGAAA GTATGGTCGA AAACGAACTA AGGTAACCAC
19501  ATAGAACCAG GTACTTTTCT ATGTGGAATC AGGCTGTTGA CAGCTATGAT CCAGATGTTA GAATTATTGA AAATCATGGA ACTGAAGATG AACTTCCAAA
       TATCTTGGTC CATGAAAAGA TACACCTTAG TCCGACAACT GTCGATACTA GGTCTACAAT CTTAATAACT TTTAGTACCT TGACTTCTAC TTGAAGGTTT
19601  TTACTGCTTT CCACTGGGAG GTGTGATTAA TACAGAGACT CTTACCAAGG TAAAACCTAA AACAGGTCAG GAAAATGGAT GGGAAAAAGA TGCTACAGAA
       AATGACGAAA GGTGACCCTC CACACTAATT ATGTCTCTGA GAATGGTTCC ATTTTGGATT TTGTCCAGTC CTTTTACCTA CCCTTTTTCT ACGATGTCTT
19701  TTTTCAGATA AAAATGAAAT AAGAGTTGGA AATAATTTTG CCATGGAAAT CAATCTAAAT GCCAACCTGT GGAGAAATTT CCTGTACTCC AACATAGCGC
       AAAAGTCTAT TTTTACTTTA TTCTCAACCT TTATTAAAAC GGTACCTTTA GTTAGATTTA CGGTTGGACA CCTCTTTAAA GGACATGAGG TTGTATCGCG
19801  TGTATTTGCC CGACAAGCTA AAGTACAGTC CTTCCAACGT AAAAATTTCT GATAACCCAA ACACCTACGA CTACATGAAC AAGCGAGTGG TGGCTCCCGG
       ACATAAACGG GCTGTTCGAT TTCATGTCAG GAAGGTTGCA TTTTTAAAGA CTATTGGGTT TGTGGATGCT GATGTACTTG TTCGCTCACC ACCGAGGGCC
19901  GCTAGTGGAC TGCTACATTA ACCTTGGAGC ACGCTGGTCC CTTGACTATA TGGACAACGT CAACCCATTT AACCACCACC GCAATGCTGG CCTGCGCTAC
       CGATCACCTG ACGATGTAAT TGGAACCTCG TGCGACCAGG GAACTGATAT ACCTGTTGCA GTTGGGTAAA TTGGTGGTGG CGTTACGACC GGACGCGATG
20001  CGCTCAATGT TGCTGGCCAA TGGTCGCTAT GTGCCCTTCC ACATCCAGGT GCCTCAGAAG TTCTTTGCCA TTAAAAACCT CCTTCTCCTG CCGGGCTCAT
       GCGAGTTACA ACGACCGGTT ACCAGCGATA CACGGGAAGG TGTAGGTCCA CGGAGTCTTC AAGAAACGGT AATTTTTGGA GGAAGAGGAC GGCCCGAGTA
                                                  PstI
20101  ACACCTACGA GTGGAACTTC AGGAAGGATG TTAACATGGT TCTGCAGAGC TCCCTAGGAA ATGACCTAAG GGTTGACGGA GCCAGCATTA AGTTTGATAG
       TGTGGATGCT CACCTTGAAG TCCTTCCTAC AATTGTACCA AGACGTCTCG AGGGATCCTT TACTGGATTC CCAACTGCCT CGGTCGTAAT TCAAACTATC
20201  CATTTGCCTT TACGCCACCT TCTTCCCCAT GGCCCACAAC ACCGCCTCCA CGCTTGAGGC CATGCTTAGA AACGACACCA ACGACCAGTC CTTTAACGAC
       GTAAACGGAA ATGCGGTGGA AGAAGGGGTA CCGGGTGTTG TGGCGGAGGT GCGAACTCCG GTACGAATCT TTGCTGTGGT TGCTGGTCAG GAAATTGCTG
20301  TATCTCTCCG CCGCCAACAT GCTCTACCCT ATACCCGCCA ACGCTACCAA CGTGCCCATA TCCATCCCCT CCCGCAACTG GGCGGCTTTC CGCGGCTGGG
       ATAGAGAGGC GGCGGTTGTA CGAGATGGGA TATGGGCGGT TGCGATGGTT GCACGGGTAT AGGTAGGGGA GGGCGTTGAC CCGCCGAAAG GCGCCGACCC
20401  CCTTCACGCG CCTTAAGACT AAGGAAACCC CATCACTGGG CTCGGGCTAC GACCCTTATT ACACCTACTC TGGCTCTATA CCCTACCTAG ATGGAACCTT
       GGAAGTGCGC GGAATTCTGA TTCCTTTGGG GTAGTGACCC GAGCCCGATG CTGGGAATAA TGTGGATGAG ACCGAGATAT GGGATGGATC TACCTTGGAA
20501  TTACCTCAAC CACACCTTTA AGAAGGTGGC CATTACCTTT GACTCTTCTG TCAGCTGGCC TGGCAATGAC CGCCTGCTTA CCCCCAACGA GTTTGAAATT
       AATGGAGTTG GTGTGGAAAT TCTTCCACCG GTAATGGAAA CTGAGAAGAC AGTCGACCGG ACCGTTACTG GCGGACGAAT GGGGGTTGCT CAAACTTTAA
20601  AAGCGCTCAG TTGACGGGGA GGGTTACAAC GTTGCCCAGT GTAACATGAC CAAAGACTGG TTCCTGGTAC AAATGCTAGC TAACTATAAC ATTGGCTACC
       TTCGCGAGTC AACTGCCCCT CCCAATGTTG CAACGGGTCA CATTGTACTG GTTTCTGACC AAGGACCATG TTTACGATCG ATTGATATTG TAACCGATGG
20701  AGGGCTTCTA TATCCCAGAG AGCTACAAGG ACCGCATGTA CTCCTTCTTT AGAAACTTCC AGCCCATGAG CCGTCAGGTG GTGGATGATA CTAAATACAA
       TCCCGAAGAT ATAGGGTCTC TCGATGTTCC TGGCGTACAT GAGGAAGAAA TCTTTGAAGG TCGGGTACTC GGCAGTCCAC CACCTACTAT GATTTATGTT
20801  GGACTACCAA CAGGTGGGCA TCCTACACCA ACACAACAAC TCTGGATTTG TTGGCTACCT TGCCCCCACC ATGCGCGAAG GACAGGCCTA CCCTGCTAAC
       CCTGATGGTT GTCCACCCGT AGGATGTGGT TGTGTTGTTG AGACCTAAAC AACCGATGGA ACGGGGGTGG TACGCGCTTC CTGTCCGGAT GGGACGATTG
                                                                       PvuI
20901  TTCCCCTATC CGCTTATAGG CAAGACCGCA GTTGACAGCA TTACCCAGAA AAAGTTTCTT TGCGATCGCA CCCTTTGGCG CATCCCATTC TCCAGTAACT
       AAGGGGATAG GCGAATATCC GTTCTGGCGT CAACTGTCGT AATGGGTCTT TTTCAAAGAA ACGCTAGCGT GGGAAACCGC GTAGGGTAAG AGGTCATTGA
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Figure 15M

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                                                                                                           BamHI
21001  TTATGTCCAT GGGCGCACTC ACAGACCTGG GCCAAAACCT TCTCTACGGC AACTCCGCCC ACGCGCTAGA CATGACTTTT GAGGTGGATC CCATGGACGA
       AATACAGGTA CCCGCGTGAG TGTCTGGACC CGGTTTTGGA AGAGATGCCG TTGAGGCGGG TGCGCGATCT GTACTGAAAA CTCCACCTAG GGTACCTGCT
21101  GCCCACCCTT CTTTATGTTT TGTTTGAAGT CTTTGACGTG GTCCGTGTGC ACCAGCCGCA CCGCGGCGTC ATCGAAACCG TGTACCTGCG CACGCCCTT
       CGGGTGGGAA GAAATACAAA ACAAACTTCA GAAACTGCAC CAGGCACACG TGGTCGGCGT GGCGCCGCAG TAGCTTTGGC ACATGGACGC GTGCGGGA
                                                                                                                  BglII
21201  TCGGCCGGCA ACGCCACAAC ATAAAGAAGC AAGCAACATC AACAACAGCT GCCGCCATGG GCTCCAGTGA GCAGGAACTG AAAGCCATTG TCAAAGATCT
       AGCCGGCCGT TGCGGTGTTG TATTTCTTCG TTCGTTGTAG TTGTTGTCGA CGGCGGTACC CGAGGTCACT CGTCCTTGAC TTTCGGTAAC AGTTTCTAGA
21301  TGGTTGTGGG CCATATTTTT TGGGCACCTA TGACAAGCGC TTTCCAGGCT TTGTTTCTCC ACACAAGCTC GCCTGCGCCA TAGTCAATAC GGCCGGTC
       ACCAACACCC GGTATAAAAA ACCCGTGGAT ACTGTTCGCG AAAGGTCCGA AACAAAGAGG TGTGTTCGAG CGGACGCGGT ATCAGTTATG CCGGCCAG
21401  GAGACTGGGG GCGTACACTG GATGGCCTTT GCCTGGAACC CGCACTCAAA AACATGCTAC CTCTTTGAGC CCTTTGGCTT TTCTGACCAG CGACTCAAGC
       CTCTGACCCC CGCATGTGAC CTACCGGAAA CGGACCTTGG GCGTGAGTTT TTGTACGATG GAGAAACTCG GGAAACCGAA AAGACTGGTC GCTGAGTTCG
21501  AGGTTTACCA GTTTGAGTAC GAGTCACTCC TGCGCCGTAG CGCCATTGCT TCTTCCCCCG ACCGCTGTAT AACGCTGGAA AAGTCCACCC AAAGCGTAC
       TCCAAATGGT CAAACTCATG CTCAGTGAGG ACGCGGCATC GCGGTAACGA AGAAGGGGGC TGGCGACATA TTGCGACCTT TTCAGGTGGG TTTCGCATGT
21601  GGGGCCCAAC TCGGCCGCCT GTGGACTATT CTGCTGCATG TTTCTCCACG CCTTTGCCAA CTGGGCCCAA ACTCCCATGG ATCACAACCC CACCATGAA
       CCCCGGGTTG AGCCGGCGGA CACCTGATAA GACGACGTAC AAAGAGGTGC GGAAACGGTT GACCCGGGTT TGAGGGTACC TAGTGTTGGG GTGGTACTT
                   KpnI
21701  CTTATTACCG GGGTACCCAA CTCCATGCTC AACAGTCCCC AGGTACAGCC CACCCTGCGT CGCAACCAGG AACAGCTCTA CAGCTTCCTG GAGCGCCAC
       GAATAATGGC CCCATGGGTT GAGGTACGAG TTGTCAGGGG TCCATGTCGG GTGGGACGCA GCGTTGGTCC TTGTCGAGAT GTCGAAGGAC CTCGCGGTG
21801  CGCCCTACTT CCGCAGCCAC AGTGCGCAGA TTAGGAGCGC CACTTCTTTT TGTCACTTGA AAAACATGTA AAAATAATGT ACTAGAGACA CTTTCAATAA
       GCGGGATGAA GGCGTCGGTG TCACGCGTCT AATCCTCGCG GTGAAGAAAA ACAGTGAACT TTTTGTACAT TTTTATTACA TGATCTCTGT GAAAGTTATT
21901  AGGCAAATGC TTTTATTTGT ACACTCTCGG GTGATTATTT ACCCCCACCC TTGCCGTCTG CGCCGTTTAA AAATCAAAGG GGTTCTGCCG CGCATCGCTA
       TCCGTTTACG AAAATAAACA TGTGAGAGCC CACTAATAAA TGGGGGTGGG AACGGCAGAC GCGGCAAATT TTTAGTTTCC CCAAGACGGC GCGTAGCGAT
22001  TGCGCCACTG GCAGGGACAC GTTGCGATAC TGGTGTTTAG TGCTCCACTT AAACTCAGGC ACAACCATCC GCGGCAGCTC GGTGAAGTTT TCACTCCACA
       ACGCGGTGAC CGTCCCTGTG CAACGCTATG ACCACAAATC ACGAGGTGAA TTTGAGTCCG TGTTGGTAGG CGCCGTCGAG CCACTTCAAA AGTGAGGTGT
                                                 EcoRV
22101  GGCTGCGCAC CATCACCAAC GCGTTTAGCA GGTCGGGCGC CGATATCTTG AAGTCGCAGT TGGGGCCTCC GCCCTGCGCG CGCGAGTTGC GATACACAGG
       CCGACGCGTG GTAGTGGTTG CGCAAATCGT CCAGCCCGCG GCTATAGAAC TTCAGCGTCA ACCCCGGAGG CGGGACGCGC GCGCTCAACG CTATGTGTCC
22201  GTTGCAGCAC TGGAACACTA TCAGCGCCGG GTGGTGCACG CTGGCCAGCA CGCTCTTGTC GGAGATCAGA TCCGCGTCCA GGTCCTCCGC GTTGCTCAGG
       CAACGTCGTG ACCTTGTGAT AGTCGCGGCC CACCACGTGC GACCGGTCGT GCGAGAACAG CCTCTAGTCT AGGCGCAGGT CCAGGAGGCG CAACGAGTCC
22301  GCGAACGGAG TCAACTTTGG TAGCTGCCTT CCCAAAAAGG GCGCGTGCCC AGGCTTTGAG TTGCACTCGC ACCGTAGTGG CATCAAAAGG TGACCGTGCC
       CGCTTGCCTC AGTTGAAACC ATCGACGGAA GGGTTTTTCC CGCGCACGGG TCCGAAACTC AACGTGAGCG TGGCATCACC GTAGTTTTCC ACTGGCACGG
22401  CGGTCTGGGC GTTAGGATAC AGCGCCTGCA TAAAAGCCTT GATCTGCTTA AAAGCCACCT GAGCCTTTGC GCCTTCAGAG AAGAACATGC CGCAAGACTT
       GCCAGACCCG CAATCCTATG TCGCGGACGT ATTTTCGGAA CTAGACGAAT TTTCGGTGGA CTCGGAAACG CGGAAGTCTC TTCTTGTACG    TTCTGAA
                        SfiI                                             BglII
22501  GCCGGAAAAC TGATTGGCCG GACAGGCCGC GTCGTGCACG CAGCACCTTG CGTCGGTGTT GGAGATCTGC ACCACATTTC GGCCCCACCG GTTCTTCACG
       CGGCCTTTTG ACTAACCGGC CTGTCCGGCG CAGCACGTGC GTCGTGGAAC GCAGCCACAA CCTCTAGACG TGGTGTAAAG CCGGGGTGGC CAAGAAGTGC
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Figure 15N

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22601  ATCTTGGCCT TGCTAGACTG CTCCTTCAGC GCGGCGCTGC CGTTTTCGGT CGTCACATCC ATTTCAATCA CGTGCTCCTT ATTTATCATA ATGCTTCCGT
       TAGAACCGGA ACGATCTGAC GAGGAAGTCG CGCCGCGACG GCAAAAGCCA GCAGTGTAGG TAAAGTTAGT GCACGAGGAA TAAATAGTAT TACGAAGGCA
                                                                                                             PstI
22701  GTAGACACTT AAGCTCGCCT TCGATCTCAG CGCAGCGGTG CAGCCACAAC GCGCAGCCCG TGGGCTCGTC ATGCTTGTAG GTCACCTCTG CAAACGACTG
       CATCTGTGAA TTCGAGCGGA AGCTAGAGTC GCGTCGCCAC GTCGGTGTTG CGCGTCGGGC ACCCGAGCAG TACGAACATC CAGTGGAGAC GTTTGCTGAC
       PstI       PstI
22801  CAGGTACGCC TGCAGGAATC GCCCCATCAT CGTCACAAAG GTCTTGTTGC TGGTGAAGGT CAGCTGCAAC CCGCGGTGCT CCTCGTTCAG CCAGGTCTTG
       GTCCATGCGG ACGTCCTTAG CGGGGTAGTA GCAGTGTTTC CAGAACAACG ACCACTTCCA GTCGACGTTG GGCGCCACGA GGAGCAAGTC GGTCCAGAAC
22901  CATACGGCCG CCAGAGCTTC CACTTGGTCA GGCAGTAGTT TGAAGTTCGC CTTTAGATCG TTATCCACGT GGTACTTGTC CATCAGCGCG CGCGCAGCCT
       GTATGCCGGC GGTCTCGAAG GTGAACCAGT CCGTCATCAA ACTTCAAGCG GAAATCTAGC AATAGGTGCA CCATGAACAG GTAGTCGCGC GCGCGTCGGA
                             PvuI
23001  CCATGCCCTT CTCCCACGCA GACACGATCG GCACACTCAG CGGGTTCATC ACCGTAATTT CACTTTCCGC TTCGCTGGGC TCTTCCTCTT CCTCTTGCGT
       GGTACGGGAA GAGGGTGCGT CTGTGCTAGC CGTGTGAGTC GCCCAAGTAG TGGCATTAAA GTGAAAGGCG AAGCGACCCG AGAAGGAGAA GGAGAACGCA
23101  CCGCATACCA CGCGCCACTG GGTCGTCTTC ATTCAGCCGC CGCACTGTGC GCTTACCTCC TTTGCCATGC TTGATTAGCA CCGGTGGGTT GCTGAAACCC
       GGCGTATGGT GCGCGGTGAC CCAGCAGAAG TAAGTCGGCG GCGTGACACG CGAATGGAGG AAACGGTACG AACTAATCGT GGCCACCCAA CGACTTTGGG
23201  ACCATTTGTA GCGCCACATC TTCTCTTTCT TCCTCGCTGT CCACGATTAC CTCTGGTGAT GGCGGGCGCT CGGGCTTGGG AGAAGGGCGC TTCTTTTTCT
       TGGTAAACAT CGCGGTGTAG AAGAGAAAGA AGGAGCGACA GGTGCTAATG GAGACCACTA CCGCCCGCGA GCCCGAACCC TCTTCCCGCG AAGAAAAAGA
23301  TCTTGGGCGC AATGGCCAAA TCCGCCGCCG AGGTCGATGG CCGCGGGCTG GGTGTGCGCG GCACCAGCGC GTCTTGTGAT GAGTCTTCCT CGTCCTCGGA
       AGAACCCGCG TTACCGGTTT AGGCGGCGGC TCCAGCTACC GGCGCCCGAC CCACACGCGC CGTGGTCGCG CAGAACACTA CTCAGAAGGA GCAGGAGCCT
23401  CTCGATACGC CGCCTCATCC GCTTTTTTGG GGGCGCCCGG GGAGGCGGCG GCGACGGGGA CGGGGACGAC ACGTCCTCCA TGGTTGGGGG ACGTCGCGCC
       GAGCTATGCG GCGGAGTAGG CGAAAAAACC CCCGCGGGCC CCTCCGCCGC CGCTGCCCCT GCCCCTGCTG TGCAGGAGGT ACCAACCCCC TGCAGCGCG
23501  GCACCGCGTC CGCGCTCGGG GGTGGTTTCG CGCTGCTCCT CTTCCCGACT GGCCATTTCC TTCTCCTATA GGCAGAAAAA GATCATGGAG TCAGTCGAGA
       CGTGGCGCAG GCGCGAGCCC CCACCAAAGC GCGACGAGGA GAAGGGCTGA CCGGTAAAGG AAGAGGATAT CCGTCTTTTT CTAGTACCTC AGTCAGCTCT
23601  AGAAGGACAG CCTAACCGCC CCCTCTGAGT TCGCCACCAC CGCCTCCACC GATGCCGCCA ACGCGCCTAC CACCTTCCCC GTCGAGGCAC CCCCGCTTGA
       TCTTCCTGTC GGATTGGCGG GGGAGACTCA AGCGGTGGTG GCGGAGGTGG CTACGGCGGT TGCGCGGATG GTGGAAGGGG CAGCTCCGTG GGGGCGAACT
23701  GGAGGAGGAA GTGATTATCG AGCAGGACCC AGGTTTTGTA AGCGAAGACG ACGAGGACCG CTCAGTACCA ACAGAGGATA AAAAGCAAGA CCAGGACAAC
       CCTCCTCCTT CACTAATAGC TCGTCCTGGG TCCAAAACAT TCGCTTCTGC TGCTCCTGGC GAGTCATGGT TGTCTCCTAT TTTTCGTTCT GGTCCTGTTG
                                                                                                  PstI
23801  GCAGAGGCAA ACGAGGAACA AGTCGGGCGG GGGGACGAAA GGCATGGCGA CTACCTAGAT GTGGGAGACG ACGTGCTGTT GAAGCATCTG CAGCGCCAGT
       CGTCTCCGTT TGCTCCTTGT TCAGCCCGCC CCCCTGCTTT CCGTACCGCT GATGGATCTA CACCCTCTGC TGCACGACAA CTTCGTAGAC GTCGCGGTCA
23901  GCGCCATTAT CTGCGACGCG TTGCAAGAGC GCAGCGATGT GCCCCTCGCC ATAGCGGATG TCAGCCTTGC CTACGAACGC CACCTATTCT CACCGCGCGT
       CGCGGTAATA GACGCTGCGC AACGTTCTCG CGTCGCTACA CGGGGAGCGG TATCGCCTAC AGTCGGAACG GATGCTTGCG GTGGATAAGA GTGGCGCGCA
24001  ACCCCCCAAA CGCCAAGAAA ACGGCACATG CGAGCCCAAC CCGCGCCTCA ACTTCTACCC CGTATTTGCC GTGCCAGAGG TGCTTGCCAC CTATCACATC
       TGGGGGGTTT GCGGTTCTTT TGCCGTGTAC GCTCGGGTTG GGCGCGGAGT TGAAGATGGG GCATAAACGG CACGGTCTCC ACGAACGGTG GATAGTGTAG
                                                                                                             EcoRV
24101  TTTTTCCAAA ACTGCAAGAT ACCCCTATCC TGCCGTGCCA ACCGCAGCCG AGCGGACAAG CAGCTGGCCT TGCGGCAGGG CGCTGTCATA CCTGATATCG
       AAAAAGGTTT TGACGTTCTA TGGGGATAGG ACGGCACGGT TGGCGTCGGC TCGCCTGTTC GTCGACCGGA ACGCCGTCCC GCGACAGTAT GGACTATAGC
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Figure 15D

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24201  CCTCGCTCAA CGAAGTGCCA AAAATCTTTG AGGGTCTTGG ACGCGACGAG AAGCGCGCGG CAAACGCTCT GCAACAGGAA AACAGCGAAA ATGAAAGTCA
       GGAGCGAGTT GCTTCACGGT TTTTAGAAAC TCCCAGAACC TGCGCTGCTC TTCGCGCGCC GTTTGCGAGA CGTTGTCCTT TTGTCGCTTT TACTTTCAGT
                              XhoI
24301  CTCTGGAGTG TTGGTGGAAC TCGAGGGTGA CAACGCGCGC CTAGCCGTAC TAAAACGCAG CATCGAGGTC ACCCACTTTG CCTACCCGGC ACTTAACCTA
       GAGACCTCAC AACCACCTTG AGCTCCCACT GTTGCGCGCG GATCGGCATG ATTTTGCGTC GTAGCTCCAG TGGGTGAAAC GGATGGGCCG TGAATTGGAT
24401  CCCCCCAAGG TCATGAGCAC AGTCATGAGT GAGCTGATCG TGCGCCGTGC GCAGCCCCTG GAGAGGGATG CAAATTTGCA AGAACAAACA GAGGAGGGCC
       GGGGGGTTCC AGTACTCGTG TCAGTACTCA CTCGACTAGC ACGCGGCACG CGTCGGGGAC CTCTCCCTAC GTTTAAACGT TCTTGTTTGT CTCCTCCCGG
24501  TACCCGCAGT TGGCGACGAG CAGCTAGCGC GCTGGCTTCA AACGCGCGAG CCTGCCGACT TGGAGGAGCG ACGCAAACTA ATGATGGCCG CAGTGCTCGT
       ATGGGCGTCA ACCGCTGCTC GTCGATCGCG CGACCGAAGT TTGCGCGCTC GGACGGCTGA ACCTCCTCGC TGCGTTTGAT TACTACCGGC GTCACGAGCA
                              SphI
24601  TACCGTGGAG CTTGAGTGCA TGCAGCGGTT CTTTGCTGAC CCGGAGATGC AGCGCAAGCT AGAGGAAACA TTGCACTACA CCTTTCGACA GGGCTACGTA
       ATGGCACCTC GAACTCACGT ACGTCGCCAA GAAACGACTG GGCCTCTACG TCGCGTTCGA TCTCCTTTGT AACGTGATGT GGAAAGCTGT CCCGATGCAT
                  BglII
24701  CGCCAGGCCT GCAAGATCTC CAACGTGGAG CTCTGCAACC TGGTCTCCTA CCTTGGAATT TTGCACGAAA ACCGCCTTGG GCAAAACGTG CTTCATTCCA
       GCGGTCCGGA CGTTCTAGAG GTTGCACCTC GAGACGTTGG ACCAGAGGAT GGAACCTTAA AACGTGCTTT TGGCGGAACC CGTTTTGCAC GAAGTAAGGT
                  AscI
24801  CGCTCAAGGG CGAGGCGCGC CGCGACTACG TCCGCGACTG CGTTTACTTA TTTCTATGCT ACACCTGGCA GACGGCCATG GGCGTTTGGC AGCAGTGCTT
       GCGAGTTCCC GCTCCGCGCG GCGCTGATGC AGGCGCTGAC GCAAATGAAT AAAGATACGA TGTGGACCGT CTGCCGGTAC CCGCAAACCG TCGTCACGAA
                                PstI
24901  GGAGGAGTGC AACCTCAAGG AGCTGCAGAA ACTGCTAAAG CAAAACTTGA AGGACCTATG GACGGCCTTC AACGAGCGCT CCGTGGCCGC GCACCTGGCG
       CCTCCTCACG TTGGAGTTCC TCGACGTCTT TGACGATTTC GTTTTGAACT TCCTGGATAC CTGCCGGAAG TTGCTCGCGA GGCACCGGCG CGTGGACCGC
25001  GACATCATTT TCCCCGAACG CCTGCTTAAA ACCCTGCAAC AGGGTCTGCC AGACTTCACC AGTCAAAGCA TGTTGCAGAA CTTTAGGAAC TTTATCCTAG
       CTGTAGTAAA AGGGGCTTGC GGACGAATTT TGGGACGTTG TCCCAGACGG TCTGAAGTGG TCAGTTTCGT ACAACGTCTT GAAATCCTTG AAATAGGATC
25101  AGCGCTCAGG AATCTTGCCC GCCACCTGCT GTGCACTTCC TAGCGACTTT GTGCCCATTA AGTACCGCGA ATGCCCTCCG CCGCTTTGGG GCCACTGCTA
       TCGCGAGTCC TTAGAACGGG CGGTGGACGA CACGTGAAGG ATCGCTGAAA CACGGGTAAT TCATGGCGCT TACGGGAGGC GGCGAAACCC CGGTGACGAT
          PstI
25201  CCTTCTGCAG CTAGCCAACT ACCTTGCCTA CCACTCTGAC ATAATGGAAG ACGTGAGCGG TGACGGTCTA CTGGAGTGTC ACTGTCGCTG CAACCTATGC
       GGAAGACGTC GATCGGTTGA TGGAACGGAT GGTGAGACTG TATTACCTTC TGCACTCGCC ACTGCCAGAT GACCTCACAG TGACAGCGAC GTTGGATACG
                                                                KpnI       PstI
25301  ACCCCGCACC GCTCCCTGGT TTGCAATTCG CAGCTGCTTA ACGAAAGTCA AATTATCGGT ACCTTTGAGC TGCAGGGTCC CTCGCCTGAC GAAAAGTCCG
       TGGGGCGTGG CGAGGGACCA AACGTTAAGC GTCGACGAAT TGCTTTCAGT TTAATAGCCA TGGAAACTCG ACGTCCCAGG GAGCGGACTG CTTTTCAGGC
25401  CGGCTCCGGG GTTGAAACTC ACTCCGGGGC TGTGGACGTC GGCTTACCTT CGCAAATTTG TACCTGAGGA CTACCACGCC CACGAGATTA GGTTCTACGA
       GCCGAGGCCC CAACTTTGAG TGAGGCCCCG ACACCTGCAG CCGAATGGAA GCGTTTAAAC ATGGACTCCT GATGGTGCGG GTGCTCTAAT CCAAGATGCT
25501  AGACCAATCC CGCCCGCCTA ATGCGGAGCT TACCGCCTGC GTCATTACCC AGGGCCACAT TCTTGGCCAA TTGCAAGCCA TCAACAAAGC CCGCCAAGAG
       TCTGGTTAGG GCGGGCGGAT TACGCCTCGA ATGGCGGACG CAGTAATGGG TCCCGGTGTA AGAACCGGTT AACGTTCGGT AGTTGTTTCG GGCGGTTCTC
25601  TTTCTGCTAC GAAAGGGACG GGGGGTTTAC TTGGACCCCC AGTCCGGCGA GGAGCTCAAC CCAATCCCCC CGCCGCCGCA GCCCTATCAG CAGCAGCCGC
       AAAGACGATG CTTTCCCTGC CCCCCAAATG AACCTGGGGG TCAGGCCGCT CCTCGAGTTG GGTTAGGGGG GCGGCGGCGT CGGGATAGTC GTCGTCGGCG
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Figure 15P

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                                                   PstI
25701  GGGCCCTTGC TTCCCAGGAT GCCACCCAAA AAGAAGCTGC AGCTGCCGCC GCCACCCACG GACGAGGAGG AATACTGGGA CAGTCAGGCA GAGGAGGTTT
       CCCGGGAACG AAGGGTCCTA CGGTGGGTTT TTCTTCGACG TCGACGGCGG CGGTGGGTGC CTGCTCCTCC TTATGACCCT GTCAGTCCGT CTCCTCCAAA
                                                              HindIII
25801  TGGACGAGGA GGAGGAGGAC ATGATGGAAG ACTGGGAGAG CCTAGACCAG GAAGCTTCCG AGGTCGAAGA GGTGTCAGAC GAAACACCGT CACCCTCGCT
       ACCTGCTCCT CCTCCTCCTG TACTACCTTC TGACCCTCTC GGATCTGGTC CTTCGAAGGC TCCAGCTTCT CCACAGTCTG CTTTGTGGCA GTGGGAGCGA
25901  CGCATTCCCC TCGCCGGCGC CCCAGAAATC GGCAACCGGT TCCAGCATGG CTACAACCTC CGCTCCTCAG GCGCCGCCGG CACTGCCCGT TCGCCGACCC
       GCGTAAGGGG AGCGGCCGCG GGGTCTTTAG CCGTTGGCCA AGGTCGTACC GATGTTGGAG GCGAGGAGTC CGCGGCGGCC GTGACGGGCA AGCGGCTGGG
26001  AACCGTAGAT GGGACACCAC TGGAACCAGG GCCGGTAAGT CCAAGCAGCC GCCGCCGTTA GCCCAAGAGC AACAACAGCG CCAAGGCTAC CGCTCATGGC
       TTGGCATCTA CCCTGTGGTG ACCTTGGTCC CGGCCATTCA GGTTCGTCGG CGGCGGCAAT CGGGTTCTCG TTGTTGTCGC GGTTCCGATG GCGAGTACCG
26101  GCGGGCACAA GAACGCCATA GTTGCTTGCT TGCAAGACTG TGGGGGCAAC ATCTCCTTCG CCCGCCGCTT TCTTCTCTAC CATCACGGCG TGGCCTTCCC
       CGCCCGTGTT CTTGCGGTAT CAACGAACGA ACGTTCTGAC ACCCCCGTTG TAGAGGAAGC GGGCGGCGAA AGAAGAGATG GTAGTGCCGC ACCGGAAGGG
26201  CCGTAACATC CTGCATTACT ACCGTCATCT CTACAGCCCA TACTGCACCG GCGGCAGCGG CAGCAACAGC AGCGGCCACA CAGAAGCAAA GGCGACCGGA
       GGCATTGTAG GACGTAATGA TGGCAGTAGA GATGTCGGGT ATGACGTGGC CGCCGTCGCC GTCGTTGTCG TCGCCGGTGT GTCTTCGTTT CCGCTGGCCT
26301  TAGCAAGACT CTGACAAAGC CCAAGAAATC CACAGCGGCG GCAGCAGCAG GAGGAGGAGC GCTGCGTCTG GCGCCCAACG AACCCGTATC GACCCGCGAG
       ATCGTTCTGA GACTGTTTCG GGTTCTTTAG GTGTCGCCGC CGTCGTCGTC CTCCTCCTCG CGACGCAGAC CGCGGGTTGC TTGGGCATAG CTGGGCGCTC
26401  CTTAGAAACA GGATTTTTCC CACTCTGTAT GCTATATTTC AACAGAGCAG GGGCCAAGAA CAAGAGCTGA AAATAAAAAA CAGGTCTCTG CGATCCCTCA
       GAATCTTTGT CCTAAAAAGG GTGAGACATA CGATATAAAG TTGTCTCGTC CCCGGTTCTT GTTCTCGACT TTTATTTTTT GTCCAGAGAC GCTAGGGAGT
26501  CCCGCAGCTG CCTGTATCAC AAAAGCGAAG ATCAGCTTCG GCGCACGCTG GAAGACGCGG AGGCTCTCTT CAGTAAATAC TGCGCGCTGA CTCTTAAGGA
       GGGCGTCGAC GGACATAGTG TTTTCGCTTC TAGTCGAAGC CGCGTGCGAC CTTCTGCGCC TCCGAGAGAA GTCATTTATG ACGCGCGACT GAGAATTCCT
26601  CTAGTTTCGC GCCCTTTCTC AAATTTAAGC GCGAAAACTA CGTCATCTCC AGCGGCCACA CCCGGCGCCA GCACCTGTTG TCAGCGCCAT TATGAGCAAG
       GATCAAAGCG CGGGAAAGAG TTTAAATTCG CGCTTTTGAT GCAGTAGAGG TCGCCGGTGT GGGCCGCGGT CGTGGACAAC AGTCGCGGTA ATACTCGTTC
26701  GAAATTCCCA CGCCCTACAT GTGGAGTTAC CAGCCACAAA TGGGACTTGC GGCTGGAGCT GCCCAAGACT ACTCAACCCG AATAAACTAC ATGAGCGCGG
       CTTTAAGGGT GCGGGATGTA CACCTCAATG GTCGGTGTTT ACCCTGAACG CCGACCTCGA CGGGTTCTGA TGAGTTGGGC TTATTTGATG TACTCGCGCC
                  EcoRV                                  EcoRI
26801  GACCCCACAT GATATCCCGG GTCAACGGAA TACGCGCCCA CCGAAACCGA ATTCTCCTGG AACAGGCGGC TATTACCACC ACACCTCGTA ATAACCTTAA
       CTGGGGTGTA CTATAGGGCC CAGTTGCCTT ATGCGCGGGT GGCTTTGGCT TAAGAGGACC TTGTCCGCCG ATAATGGTGG TGTGGAGCAT TATTGGAATT
26901  TCCCCGTAGT TGGCCCGCTG CCCTGGTGTA CCAGGAAAGT CCCGCTCCCA CCACTGTGGT ACTTCCCAGA GACGCCCAGG CCGAAGTTCA GATGACTAAC
       AGGGGCATCA ACCGGGCGAC GGGACCACAT GGTCCTTTCA GGGCGAGGGT GGTGACACCA TGAAGGGTCT CTGCGGGTCC GGCTTCAAGT CTACTGATTG
27001  TCAGGGGCGC AGCTTGCGGG CGGCTTTCGT CACAGGGTGC GGTCGCCCGG GCAGGGTATA ACTCACCTGA CAATCAGAGG GCGAGGTATT CAGCTCAACG
       AGTCCCCGCG TCGAACGCCC GCCGAAAGCA GTGTCCCACG CCAGCGGGCC CGTCCCATAT TGAGTGGACT GTTAGTCTCC CGCTCCATAA GTCGAGTTGC
27101  ACGAGTCGGT GAGCTCCTCG CTTGGTCTCC GTCCGGACGG GACATTTCAG ATCGGCGGCG CCGGCCGCTC TTCATTCACG CCTCGTCAGG CAATCCTAAC
       TGCTCAGCCA CTCGAGGAGC GAACCAGAGG CAGGCCTGCC CTGTAAAGTC TAGCCGCCGC GGCCGGCGAG AAGTAAGTGC GGAGCAGTCC GTTAGGATTG
        PstI
27201  TCTGCAGACC TCGTCCTCTG AGCCGCGCTC TGGAGGCATT GGAACTCTGC AATTTATTGA GGAGTTTGTG CCATCGGTCT ACTTTAACCC CTTCTCGGGA
       AGACGTCTGG AGCAGGAGAC TCGGCGCGAG ACCTCCGTAA CCTTGAGACG TTAAATAACT CCTCAAACAC GGTAGCCAGA TGAAATTGGG GAAGAGCCCT
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Figure 15Q

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27301  CCTCCCGGCC ACTATCCGGA TCAATTTATT CCTAACTTTG ACGCGGTAAA GGACTCGGCG GACGGCTACG ACTGAATGTT AAGTGGAGAG GCAGAGCAAC
       GGAGGGCCGG TGATAGGCCT AGTTAAATAA GGATTGAAAC TGCGCCATTT CCTGAGCCGC CTGCCGATGC TGACTTACAA TTCACCTCTC CGTCTCGTTG
27401  TGCGCCTGAA ACACCTGGTC CACTGTCGCC GCCACAAGTG CTTTGCCCGC GACTCCGGTG AGTTTTGCTA CTTTGAATTG CCCGAGGATC ATATCGAGGG
       ACGCGGACTT TGTGGACCAG GTGACAGCGG CGGTGTTCAC GAAACGGGCG CTGAGGCCAC TCAAAACGAT GAAACTTAAC GGGCTCCTAG TATAGCTCCC
27501  CCCGGCGCAC GGCGTCCGGC TTACCGCCCA GGGAGAGCTT GCCCGTAGCC TGATTCGGGA GTTTACCCAG CGCCCCCTGC TAGTTGAGCG GGACAGGGGA
       GGGCCGCGTG CCGCAGGCCG AATGGCGGGT CCCTCTCGAA CGGGCATCGG ACTAAGCCCT CAAATGGGTC GCGGGGGACG ATCAACTCGC CCTGTCCCCT
                                                          BglII
27601  CCCTGTGTTC TCACTGTGAT TTGCAACTGT CCTAACCCTG GATTACATCA AGATCTTTGT TGCCATCTCT GTGCTGAGTA TAATAAATAC AGAAATTAAA
       GGGACACAAG AGTGACACTA AACGTTGACA GGATTGGGAC CTAATGTAGT TCTAGAAACA ACGGTAGAGA CACGACTCAT ATTATTTATG TCTTTAATTT
27701  ATATACTGGG GCTCCTATCG CCATCCTGTA AACGCCACCG TCTTCACCCG CCCAAGCAAA CCAAGGCGAA CCTTACCTGG TACTTTTAAC ATCTCTCCCT
       TATATGACCC CGAGGATAGC GGTAGGACAT TTGCGGTGGC AGAAGTGGGC GGGTTCGTTT GGTTCCGCTT GGAATGGACC ATGAAAATTG TAGAGAGGGA
27801  CTGTGATTTA CAACAGTTTC AACCCAGACG GAGTGAGTCT ACGAGAGAAC CTCTCCGAGC TCAGCTACTC CATCAGAAAA AACACCACCC TCCTTACCTG
       GACACTAAAT GTTGTCAAAG TTGGGTCTGC CTCACTCAGA TGCTCTCTTG GAGAGGCTCG AGTCGATGAG GTAGTCTTTT TTGTGGTGGG AGGAATGGAC
27901  CCGGGAACGT ACGAGTGCGT CACCGGCCGC TGCACCACAC CTACCGCCTG ACCGTAAACC AGACTTTTTC CGGACAGACC TCAATAACTC TGTTTACCAG
       GGCCCTTGCA TGCTCACGCA GTGGCCGGCG ACGTGGTGTG GATGGCGGAC TGGCATTTGG TCTGAAAAAG GCCTGTCTGG AGTTATTGAG ACAAATGGTC
28001  AACAGGAGGT GAGCTTAGAA AACCCTTAGG GTATTAGGCC AAAGGCGCAG CTACTGTGGG GTTTATGAAC AATTCAAGCA ACTCTACGGG CTATTCTAAT
       TTGTCCTCCA CTCGAATCTT TTGGGAATCC CATAATCCGG TTTCCGCGTC GATGACACCC CAAATACTTG TTAAGTTCGT TGAGATGCCC GATAAGATTA
            XbaI
28101  TCAGGTTTCT CTAGAATCGG GGTTGGGGTT ATTCTCTGTC TTGTGATTCT CTTTATTCTT ATACTAACGC TTCTCTGCCT AAGGCTCGCC GCCTGCTGTG
       AGTCCAAAGA GATCTTAGCC CCAACCCCAA TAAGAGACAG AACACTAAGA GAAATAAGAA TATGATTGCG AAGAGACGGA TTCCGAGCGG CGGACGACAC
28201  TGCACATTTG CATTTATTGT CAGCTTTTTA AACGCTGGGG TCGCCACCCA AGATGATTAG GTACATAATC CTAGGTTTAC TCACCCTTGC GTCAGCCCAC
       ACGTGTAAAC GTAAATAACA GTCGAAAAAT TTGCGACCCC AGCGGTGGGT TCTACTAATC CATGTATTAG GATCCAAATG AGTGGGAACG CAGTCGGGTG
 KpnI
28301  GGTACCACCC AAAAGGTGGA TTTTAAGGAG CCAGCCTGTA ATGTTACATT CGCAGCTGAA GCTAATGAGT GCACCACTCT TATAAAATGC ACCACAGAAG
       CCATGGTGGG TTTTCCACCT AAAATTCCTC GGTCGGACAT TACAATGTAA GCGTCGACTT CGATTACTCA CGTGGTGAGA ATATTTTACG TGGTGTCTTC
28401  ATGAAAAGCT GCTTATTCGC CACAAAAACA AAATTGGCAA GTATGCTGTT TATGCTATTT GGCAGCCAGG TGACACTACA GAGTATAATG TTACAGTTTT
       TACTTTTCGA CGAATAAGCG GTGTTTTTGT TTTAACCGTT CATACGACAA ATACGATAAA CCGTCGGTCC ACTGTGATGT CTCATATTAC AATGTCAAAA
                              Bst1107I
28501  CCAGGGTAAA AGTCATAAAA CTTTTATGTA TACTTTTCCA TTTTATGAAA TGTGCGACAT TACCATGTAC ATGAGCAAAC AGTATAAGTT GTGGCCCCCA
       GGTCCCATTT TCAGTATTTT GAAAATACAT ATGAAAAGGT AAAATACTTT ACACGCTGTA ATGGTACATG TACTCGTTTG TCATATTCAA CACCGGGGGT
28601  CAAAATTGTG TGGAAAACAC TGGCACTTTC TGCTGCACTG CTATGCTAAT TACAGTGCTC GCTTTGGTCT GTACCCTACT CTATATTAAA TACAAAAGCA
       GTTTTAACAC ACCTTTTGTG ACCGTGAAAG ACGACGTGAC GATACGATTA ATGTCACGAG CGAAACCAGA CATGGGATGA GATATAATTT ATGTTTTCGT
28701  GACGCAGCTT TATTGAGGAA AAGAAAATGC CTTAATTTAC TAAGTTACAA AGCTAATGTC ACCACTAACT GCTTTACTCG CTGCTTGCAA AACAAATTGA
       CTGCGTCGAA ATAACTCCTT TTCTTTTACG GAATTAAATG ATTCAATGTT TCGATTACAG TGGTGATTGA CGAAATGAGC GACGAACGTT TTGTTTAACT
28801  AAAAGTTAGC ATTATAATTA GAATAGGATT TAAACCCCCC GGTCATTTCC TGCTCAATAC CATTCCCCTG AACAATTGAC TCTATGTGGG ATATGCTCCA
       TTTTCAATCG TAATATTAAT CTTATCCTAA ATTTGGGGGG CCAGTAAAGG ACGAGTTATG GTAAGGGGAC TTGTTAACTG AGATACACCC TATACGAGGT
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Figure 15R

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28901  GCGCTACAAC CTTGAAGTCA GGCTTCCTGG ATGTCAGCAT CTGACTTTGG CCAGCACCTG TCCCGCGGAT TTGTTCCAGT CCAACTACAG CGACCCACCC
       CGCGATGTTG GAACTTCAGT CCGAAGGACC TACAGTCGTA GACTGAAACC GGTCGTGGAC AGGGCGCCTA AACAAGGTCA GGTTGATGTC GCTGGGTGGG
29001  TAACAGAGAT GACCAACACA ACCAACGCGG CCGCCGCTAC CGGACTTACA TCTACCACAA ATACACCCCA AGTTTCTGCC TTTGTCAATA ACTGGGATAA
       ATTGTCTCTA CTGGTTGTGT TGGTTGCGCC GGCGGCGATG GCCTGAATGT AGATGGTGTT TATGTGGGGT TCAAAGACGG AAACAGTTAT TGACCCTATT
29101  CTTGGGCATG TGGTGGTTCT CCATAGCGCT TATGTTTGTA TGCCTTATTA TTATGTGGCT CATCTGCTGC CTAAAGCGCA AACGCGCCCG ACCACCCATC
       GAACCCGTAC ACCACCAAGA GGTATCGCGA ATACAAACAT ACGGAATAAT AATACACCGA GTAGACGACG GATTTCGCGT TTGCGCGGGC TGGTGGGTAG
29201  TATAGTCCCA TCATTGTGCT ACACCCAAAC AATGATGGAA TCCATAGATT GGACGGACTG AAACACATGT TCTTTTCTCT TACAGTATGA TTAAATGAGA
       ATATCAGGGT AGTAACACGA TGTGGGTTTG TTACTACCTT AGGTATCTAA CCTGCCTGAC TTTGTGTACA AGAAAAGAGA ATGTCATACT AATTTACTCT
                  XhoI
29301  CATGATTCCT CGAGTTTTTA TATTACTGAC CCTTGTTGCG CTTTTTTGTG CGTGCTCCAC ATTGGCTGCG GTTTCTCACA TCGAAGTAGA CTGCATTCCA
       GTACTAAGGA GCTCAAAAAT ATAATGACTG GGAACAACGC GAAAAAACAC GCACGAGGTG TAACCGACGC CAAAGAGTGT AGCTTCATCT GACGTAAGGT
                                                             PstI
29401  GCCTTCACAG TCTATTTGCT TTACGGATTT GTCACCCTCA CGCTCATCTG CAGCCTCATC ACTGTGGTCA TCGCCTTTAT CCAGTGCATT GACTGGGTCT
       CGGAAGTGTC AGATAAACGA AATGCCTAAA CAGTGGGAGT GCGAGTAGAC GTCGGAGTAG TGACACCAGT AGCGGAAATA GGTCACGTAA CTGACCCAGA
                                                                              EcoRI
29501  GTGTGCGCTT TGCATATCTC AGACACCATC CCCAGTACAG GGACAGGACT ATAGCTGAGC TTCTTAGAAT TCTTTAATTA TGAAATTTAC TGTGACTTTT
       CACACGCGAA ACGTATAGAG TCTGTGGTAG GGGTCATGTC CCTGTCCTGA TATCGACTCG AAGAATCTTA AGAAATTAAT ACTTTAAATG ACACTGAAAA
29601  CTGCTGATTA TTTGCACCCT ATCTGCGTTT TGTTCCCCGA CCTCCAAGCC TCAAAGACAT ATATCATGCA GATTCACTCG TATATGGAAT ATTCCAAGTT
       GACGACTAAT AAACGTGGGA TAGACGCAAA ACAAGGGGCT GGAGGTTCGG AGTTTCTGTA TATAGTACGT CTAAGTGAGC ATATACCTTA TAAGGTTCAA
                                                                         PstI
29701  GCTACAATGA AAAAAGCGAT CTTTCCGAAG CCTGGTTATA TGCAATCATC TCTGTTATGG TGTTCTGCAG TACCATCTTA GCCCTAGCTA TATATCCCTA
       CGATGTTACT TTTTTCGCTA GAAAGGCTTC GGACCAATAT ACGTTAGTAG AGACAATACC ACAAGACGTC ATGGTAGAAT CGGGATCGAT ATATAGGGAT
29801  CCTTGACATT GGCTGGAACG CAATAGATGC CATGAACCAC CCAACTTTCC CCGCGCCCGC TATGCTTCCA CTGCAACAAG TTGTTGCCGG CGGCTTTGTC
       GGAACTGTAA CCGACCTTGC GTTATCTACG GTACTTGGTG GGTTGAAAGG GGCGCGGGCG ATACGAAGGT GACGTTGTTC AACAACGGCC GCCGAAACAG
                                                                                                    XbaI
                                                                                                  BglII
29901  CCAGCCAATC AGCCTCGCCC ACCTTCTCCC ACCCCCACTG AAATCAGCTA CTTTAATCTA ACAGGAGGAG ATGACTGACA CCCTAGATCT AGAAATGGAC
       GGTCGGTTAG TCGGAGCGGG TGGAAGAGGG TGGGGGTGAC TTTAGTCGAT GAAATTAGAT TGTCCTCCTC TACTGACTGT GGGATCTAGA TCTTTACCTG
30001  GGAATTATTA CAGAGCAGCG CCTGCTAGAA AGACGCAGGG CAGCGGCCGA GCAACAGCGC ATGAATCAAG AGCTCCAAGA CATGGTTAAC TTGCACCAGT
       CCTTAATAAT GTCTCGTCGC GGACGATCTT TCTGCGTCCC GTCGCCGGCT CGTTGTCGCG TACTTAGTTC TCGAGGTTCT GTACCAATTG AACGTGGTCA
30101  GCAAAAGGGG TATCTTTTGT CTCGTAAAGC AGGCCAAAGT CACCTACGAC AGTAATACCA CCGGACACCG CCTTAGCTAC AAGTTGCCAA CCAAGCGTCA
       CGTTTTCCCC ATAGAAAACA GAGCATTTCG TCCGGTTTCA GTGGATGCTG TCATTATGGT GGCCTGTGGC GGAATCGATG TTCAACGGTT GGTTCGCAGT
30201  GAAATTGGTG GTCATGGTGG CAGAAAAGCC CATTACCATA ACTCAGCACT CGGTAGAAAC CGAAGGCTGC ATTCACTCAC CTTGTCAAGG ACCTGAGGAT
       CTTTAACCAC CAGTACCACC CTCTTTTCGG GTAATGGTAT TGAGTCGTGA GCCATCTTTG GCTTCCGACG TAAGTGAGTG GAACAGTTCC TGGACTCCTA
                                             BglII
30301  CTCTGCACCC TTATTAAGAC CCTGTGCGGT CTCAAAGATC TTATTCCCTT TAACTAATAA AAAAAAATAA TAAAGCATCA CTTACTTAAA ATCAGTTAGC
       GAGACGTGGG AATAATTCTG GGACACGCCA GAGTTTCTAG AATAAGGGAA ATTGATTATT TTTTTTTATT ATTTCGTAGT GAATGAATTT TAGTCAATCG
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Figure 15S

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30401 AAATTTCTGT CCAGTTTATT CAGCAGCACC TCCTTGCCCT CCTCCCAGCT CTGGTATTGC AGCTTCCTCC TGGCTGCAAA CTTTCTCCAC AATCTAAATG
      TTTAAAGACA GGTCAAATAA GTCGTCGTGG AGGAACGGGA GGAGGGTCGA GACCATAACG TCGAAGGAGG ACCGACGTTT GAAAGAGGTG TTAGATTTAC
30501 GAATGTCAGT TTCCTCCTGT TCCTGTCCAT CCGCACCCAC TATCTTCATG TTGTTGCAGA TGAAGCGCGC AAGACCGTCT GAAGATACCT TCAACCCCGT
      CTTACAGTCA AAGGAGGACA AGGACAGGTA GGCGTGGGTG ATAGAAGTAC AACAACGTCT ACTTCGCGCG TTCTGGCAGA CTTCTATGGA AGTTGGGGCA
30601 GTATCCATAT GACACGGAAA CCGGTCCTCC AACTGTGCCT TTTCTTACTC CTCCCTTTGT ATCCCCCAAT GGGTTTCAAG AGAGTCCCCC TGGGGTACTC
      CATAGGTATA CTGTGCCTTT GGCCAGGAGG TTGACACGGA AAAGAATGAG GAGGGAAACA TAGGGGGTTA CCCAAAGTTC TCTCAGGGGG ACCCCATGAG
                                         SphI
30701 TCTTTGCGCC TATCCGAACC TCTAGTTACC TCCAATGGCA TGCTTGCGCT CAAAATGGGC AACGGCCTCT CTCTGGACGA GGCCGGCAAC CTTACCTCCC
      AGAAACGCGG ATAGGCTTGG AGATCAATGG AGGTTACCGT ACGAACGCGA GTTTTACCCG TTGCCGGAGA GAGACCTGCT CCGGCCGTTG GAATGGAGGG
30801 AAAATGTAAC CACTGTGAGC CCACCTCTCA AAAAAACCAA GTCAAACATA AACCTGGAAA TATCTGCACC CCTCACAGTT ACCTCAGAAG CCCTAACTGT
      TTTTACATTG GTGACACTCG GGTGGAGAGT TTTTTTGGTT CAGTTTGTAT TTGGACCTTT ATAGACGTGG GGAGTGTCAA TGGAGTCTTC GGGATTGACA
30901 GGCTGCCGCC GCACCTCTAA TGGTCGCGGG CAACACACTC ACCATGCAAT CACAGGCCCC GCTAACCGTG CACGACTCCA AACTTAGCAT TGCCACCCAA
      CCGACGGCGG CGTGGAGATT ACCAGCGCCC GTTGTGTGAG TGGTACGTTA GTGTCCGGGG CGATTGGCAC GTGCTGAGGT TTGAATCGTA ACGGTGGGTT
31001 GGACCCCTCA CAGTGTCAGA AGGAAAGCTA GCCCTGCAAA CATCAGGCCC CCTCACCACC ACCGATAGCA GTACCCTTAC TATCACTGCC TCACCCCCTT
      CCTGGGGAGT GTCACAGTCT TCCTTTCGAT CGGGACGTTT GTAGTCCGGG GGAGTGGTGG TGGCTATCGT CATGGGAATG ATAGTGACGG AGTGGGGGAA
31101 TAACTACTGC CACTGGTAGC TTGGGCATTG ACTTGAAAGA GCCCATTTAT ACACAAAATG GAAAACTAGG ACTAAAGTAC GGGGCTCCTT TGCATGTAAC
      ATTGATGACG GTGACCATCG AACCCGTAAC TGAACTTTCT CGGGTAAATA TGTGTTTTAC CTTTTGATCC TGATTTCATG CCCCGAGGAA ACGTACATTG
31201 AGACGACCTA AACACTTTGA CCGTAGCAAC TGGTCCAGGT GTGACTATTA ATAATACTTC CTTGCAAACT AAAGTTACTG GAGCCTTGGG TTTTGATTCA
      TCTGCTGGAT TTGTGAAACT GGCATCGTTG ACCAGGTCCA CACTGATAAT TATTATGAAG GAACGTTTGA TTTCAATGAC CTCGGAACCC AAAACTAAGT
31301 CAAGGCAATA TGCAACTTAA TGTAGCAGGA GGACTAAGGA TTGATTCTCA AAACAGACGC CTTATACTTG ATGTTAGTTA TCCGTTTGAT GCTCAAAACC
      GTTCCGTTAT ACGTTGAATT ACATCGTCCT CCTGATTCCT AACTAAGAGT TTTGTCTGCG GAATATGAAC TACAATCAAT AGGCAAACTA CGAGTTTTGG
31401 AACTAAATCT AAGACTAGGA CAGGGCCCTC TTTTTATAAA CTCAGCCCAC AACTTGGATA TTAACTACAA CAAAGGCCTT TACTTGTTTA CAGCTTCAAA
      TTGATTTAGA TTCTGATCCT GTCCCGGGAG AAAAATATTT GAGTCGGGTG TTGAACCTAT AATTGATGTT GTTTCCGGAA ATGAACAAAT GTCGAAGTTT
                HindIII
31501 CAATTCCAAA AAGCTTGAGG TTAACCTAAG CACTGCCAAG GGGTTGATGT TTGACGCTAC AGCCATAGCC ATTAATGCAG GAGATGGGCT TGAATTTGGT
      GTTAAGGTTT TTCGAACTCC AATTGGATTC GTGACGGTTC CCCAACTACA AACTGCGATG TCGGTATCGG TAATTACGTC CTCTACCCGA ACTTAAACCA
31601 TCACCTAATG CACCAAACAC AAATCCCCTC AAAACAAAAA TTGGCCATGG CCTAGAATTT GATTCAAACA AGGCTATGGT TCCTAAACTA GGAACTGGCC
      AGTGGATTAC GTGGTTTGTG TTTAGGGGAG TTTTGTTTTT AACCGGTACC GGATCTTAAA CTAAGTTTGT TCCGATACCA AGGATTTGAT CCTTGACCGG
31701 TTAGTTTTGA CAGCACAGGT GCCATTACAG TAGGAAACAA AAATAATGAT AAGCTAACTT TGTGGACCAC ACCAGCTCCA TCTCCTAACT GTAGACTAAA
      AATCAAAACT GTCGTGTCCA CGGTAATGTC ATCCTTTGTT TTTATTACTA TTCGATTGAA ACACCTGGTG TGGTCGAGGT AGAGGATTGA CATCTGATTT
31801 TGCAGAGAAA GATGCTAAAC TCACTTTGGT CTTAACAAAA TGTGGCAGTC AAATACTTGC TACAGTTTCA GTTTTGGCTG TTAAAGGCAG TTTGGCTCCA
      ACGTCTCTTT CTACGATTTG AGTGAAACCA GAATTGTTTT ACACCGTCAG TTTATGAACG ATGTCAAAGT CAAAACCGAC AATTTCCGTC AAACCGAGGT
31901 ATATCTGGAA CAGTTCAAAG TGCTCATCTT ATTATAAGAT TTGACGAAAA TGGAGTGCTA CTAAACAATT CCTTCCTGGA CCCAGAATAT TGGAACTTTA
      TATAGACCTT GTCAAGTTTC ACGAGTAGAA TAATATTCTA AACTGCTTTT ACCTCACGAT GATTTGTTAA GGAAGGACCT GGGTCTTATA ACCTTGAAAT
                BglII
32001 GAAATGGAGA TCTTACTGAA GGCACAGCCT ATACAAACGC TGTTGGATTT ATGCCTAACC TATCAGCTTA TCCAAAATCT CACGGTAAAA CTGCCAAAAG
      CTTTACCTCT AGAATGACTT CCGTGTCGGA TATGTTTGCG ACAACCTAAA TACGGATTGG ATAGTCGAAT AGGTTTTAGA GTGCCATTTT GACGGTTTTC
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Figure 15T

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                                                                       PstI
32101  TAACATTGTC AGTCAAGTTT ACTTAAACGG AGACAAAACT AAACCTGTAA CACTAACCAT TACACTAAAC GGTACACAGG AAACAGGAGA CACAACTCCA
       ATTGTAACAG TCAGTTCAAA TGAATTTGCC TCTGTTTTGA TTTGGACATT GTGATTGGTA ATGTGATTTG CCATGTGTCC TTTGTCCTCT GTGTTGAGGT
32201  AGTGCATACT CTATGTCATT TTCATGGGAC TGGTCTGGCC ACAACTACAT TAATGAAATA TTTGCCACAT CCTCTTACAC TTTTTCATAC ATTGCCCAAT
       TCACGTATGA GATACAGTAA AAGTACCCTG ACCAGACCGG TGTTGATGTA ATTACTTTAT AAACGGTGTA GGAGAATGTG AAAAAGTATG TAACGGGTTA
32301  AATAAAGAAT CGTTTGTGTT ATGTTTCAAC GTGTTTATTT TTCAATTGCA GAAAATTTCA AGTCATTTTT CATTCAGTAG TATAGCCCCA CCACCACATA
       TTATTTCTTA GCAAACACAA TACAAAGTTG CACAAATAAA AAGTTAACGT CTTTTAAAGT TCAGTAAAAA GTAAGTCATC ATATCGGGGT GGTGGTGTAT
32401  GCTTATACAG ATCACCGTAC CTTAATCAAA CTCACAGAAC CCTAGTATTC AACCTGCCAC CTCCCTCCCA ACACACAGAG TACACAGTCC TTTCTCCCCG
       CGAATATGTC TAGTGGCATG GAATTAGTTT GAGTGTCTTG GGATCATAAG TTGGACGGTG GAGGGAGGGT TGTGTGTCTC ATGTGTCAGG AAAGAGGGGC
32501  GCTGGCCTTA AAAAGCATCA TATCATGGGT AACAGACATA TTCTTAGGTG TTATATTCCA CACGGTTTCC TGTCGAGCCA AACGCTCATC AGTGATATTT
       CGACCGGAAT TTTTCGTAGT ATAGTACCCA TTGTCTGTAT AAGAATCCAC AATATAAGGT GTGCCAAAGG ACAGCTCGGT TTGCGAGTAG TCACTATAAA
32601  ATAAACTCCC CGGGCAGCTC ACTTAAGTTC ATGTCGCTGT CCAGCTGCTG AGCCACAGGC TGCTGTCCAA CTTGCGGTTG CTTAACGGGC GGCGAAGGAG
       TATTTGAGGG GCCCGTCGAG TGAATTCAAG TACAGCGACA GGTCGACGAC TCGGTGTCCG ACGACAGGTT GAACGCCAAC GAATTGCCCG CCGCTTCCTC
                                                              PstI
32701  AAGTCCACGC CTACATGGGG GTAGAGTCAT AATCGTGCAT CAGGATAGGG CGGTGGTGCT GCAGCAGCGC GCGAATAAAC TGCTGCCGCC GCCGCTCCGT
       TTCAGGTGCG GATGTACCCC CATCTCAGTA TTAGCACGTA GTCCTATCCC GCCACCACGA CGTCGTCGCG CGCTTATTTG ACGACGGCGG CGGCGAGGCA
        PstI
32801  CCTGCAGGAA TACAACATGG CAGTGGTCTC CTCAGCGATG ATTCGCACCG CCCGCAGCAT AAGGCGCCTT GTCCTCCGGG CACAGCAGCG CACCCTGATC
       GGACGTCCTT ATGTTGTACC GTCACCAGAG GAGTCGCTAC TAAGCGTGGC GGGCGTCGTA TTCCGCGGAA CAGGAGGCCC GTGTCGTCGC GTGGGACTAG
                         PstI
32901  TCACTTAAAT CAGCACAGTA ACTGCAGCAC AGCACCACAA TATTGTTCAA AATCCCACAG TGCAAGGCGC TGTATCCAAA GCTCATGGCG GGGACCACAG
       AGTGAATTTA GTCGTGTCAT TGACGTCGTG TCGTGGTGTT ATAACAAGTT TTAGGGTGTC ACGTTCCGCG ACATAGGTTT CGAGTACCGC CCCTGGTGTC
33001  AACCCACGTG GCCATCATAC CACAAGCGCA GGTAGATTAA GTGGCGACCC CTCATAAACA CGCTGGACAT AAACATTACC TCTTTTGGCA TGTTGTAATT
       TTGGGTGCAC CGGTAGTATG GTGTTCGCGT CCATCTAATT CACCGCTGGG GAGTATTTGT GCGACCTGTA TTTGTAATGG AGAAAACCGT ACAACATTAA
             KpnI                                                                                           PstI
33101  CACCACCTCC CGGTACCATA TAAACCTCTG ATTAAACATG GCGCCATCCA CCACCATCCT AAACCAGCTG GCCAAAACCT GCCCGCCGGC TATACACTGC
       GTGGTGGAGG GCCATGGTAT ATTTGGAGAC TAATTTGTAC CGCGGTAGGT GGTGGTAGGA TTTGGTCGAC CGGTTTTGGA CGGGCGGCCG ATATGTGACG
PstI                                                                         EcoRV
33201  AGGGAACCGG GACTGGAACA ATGACAGTGG AGAGCCCAGG ACTCGTAACC ATGGATCATC ATGCTCGTCA TGATATCAAT GTTGGCACAA CACAGGCACA
       TCCCTTGGCC CTGACCTTGT TACTGTCACC TCTCGGGTCC TGAGCATTGG TACCTAGTAG TACGAGCAGT ACTATAGTTA CAACCGTGTT GTGTCCGTGT
                                                                                                        PstI
33301  CGTGCATACA CTTCCTCAGG ATTACAAGCT CCTCCCGCGT TAGAACCATA TCCCAGGGAA CAACCCATTC CTGAATCAGC GTAAATCCCA CACTGCAGGG
       GCACGTATGT GAAGGAGTCC TAATGTTCGA GGAGGGCGCA ATCTTGGTAT AGGGTCCCTT GTTGGGTAAG GACTTAGTCG CATTTAGGGT GTGACGTCCC
33401  AAGACCTCGC ACGTAACTCA CGTTGTGCAT TGTCAAAGTG TTACATTCGG GCAGCAGCGG ATGATCCTCC AGTATGGTAG CGCGGGTTTC TGTCTCAAAA
       TTCTGGAGCG TGCATTGAGT GCAACACGTA ACAGTTTCAC AATGTAAGCC CGTCGTCGCC TACTAGGAGG TCATACCATC GCGCCCAAAG ACAGAGTTTT
33501  GGAGGTAGAC GATCCCTACT GTACGGAGTG CGCCGAGACA ACCGAGATCG TGTTGGTCGT AGTGTCATGC CAAATGGAAC GCCGGACGTA GTCATATTTC
       CCTCCATCTG CTAGGGATGA CATGCCTCAC GCGGCTCTGT TGGCTCTAGC ACAACCAGCA TCACAGTACG GTTTACCTTG CGGCCTGCAT CAGTATAAAG
```

Figure 15U

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BglII

33601 CTGAAGCAAA ACCAGGTGCG GGCGTGACAA ACAGATCTGC GTCTCCGGTC TCGCCGCTTA GATCGCTCTG TGTAGTAGTT GTAGTATATC CACTCTCTCA
GACTTCGTTT TGGTCCACGC CCGCACTGTT TGTCTAGACG CAGAGGCCAG AGCGGCGAAT CTAGCGAGAC ACATCATCAA CATCATATAG GTGAGAGAGT

33701 AAGCATCCAG GCGCCCCCTG GCTTCGGGTT CTATGTAAAC TCCTTCATGC GCCGCTGCCC TGATAACATC CACCACCGCA GAATAAGCCA CACCCAGCCA
TTCGTAGGTC CGCGGGGGAC CGAAGCCCAA GATACATTTG AGGAAGTACG CGGCGACGGG ACTATTGTAG GTGGTGGCGT CTTATTCGGT GTGGGTCGGT

33801 ACCTACACAT TCGTTCTGCG AGTCACACAC GGGAGGAGCG GGAAGAGCTG GAAGAACCAT GTTTTTTTTT TTATTCCAAA AGATTATCCA AAACCTCAAA
TGGATGTGTA AGCAAGACGC TCAGTGTGTG CCCTCCTCGC CCTTCTCGAC CTTCTTGGTA CAAAAAAAAA AATAAGGTTT TCTAATAGGT TTTGGAGTTT

BglII

33901 ATGAAGATCT ATTAAGTGAA CGCGCTCCCC TCCGGTGGCG TGGTCAAACT CTACAGCCAA AGAACAGATA ATGGCATTTG TAAGATGTTG CACAATGGCT
TACTTCTAGA TAATTCACTT GCGCGAGGGG AGGCCACCGC ACCAGTTTGA GATGTCGGTT TCTTGTCTAT TACCGTAAAC ATTCTACAAC GTGTTACCGA

34001 TCCAAAAGGC AAACGGCCCT CACGTCCAAG TGGACGTAAA GGCTAAACCC TTCAGGGTGA ATCTCCTCTA TAAACATTCC AGCACCTTCA ACCATGCCCA
AGGTTTTCCG TTTGCCGGGA GTGCAGGTTC ACCTGCATTT CCGATTTGGG AAGTCCCACT TAGAGGAGAT ATTTGTAAGG TCGTGGAAGT TGGTACGGGT

34101 AATAATTCTC ATCTCGCCAC CTTCTCAATA TATCTCTAAG CAAATCCCGA ATATTAAGTC CGGCCATTGT AAAAATCTGC TCCAGAGCGC CCTCCACCTT
TTATTAAGAG TAGAGCGGTG GAAGAGTTAT ATAGAGATTC GTTTAGGGCT TATAATTCAG GCCGGTAACA TTTTTAGACG AGGTCTCGCG GGAGGTGGAA

34201 CAGCCTCAAG CAGCGAATCA TGATTGCAAA AATTCAGGTT CCTCACAGAC CTGTATAAGA TTCAAAAGCG GAACATTAAC AAAAATACCG CGATCCCGTA
GTCGGAGTTC GTCGCTTAGT ACTAACGTTT TTAAGTCCAA GGAGTGTCTG GACATATTCT AAGTTTTCGC CTTGTAATTG TTTTTATGGC GCTAGGGCAT

34301 GGTCCCTTCG CAGGGCCAGC TGAACATAAT CGTGCAGGTC TGCACGGACC AGCGCGGCCA CTTCCCCGCC AGGAACCATG ACAAAAGAAC CCACACTGAT
CCAGGGAAGC GTCCCGGTCG ACTTGTATTA GCACGTCCAG ACGTGCCTGG TCGCGCCGGT GAAGGGGCGG TCCTTGGTAC TGTTTTCTTG GGTGTGACTA

HindIII

34401 TATGACACGC ATACTCGGAG CTATGCTAAC CAGCGTAGCC CCGATGTAAG CTTGTTGCAT GGGCGGCGAT ATAAAATGCA AGGTGCTGCT CAAAAAATCA
ATACTGTGCG TATGAGCCTC GATACGATTG GTCGCATCGG GGCTACATTC GAACAACGTA CCCGCCGCTA TATTTTACGT TCCACGACGA GTTTTTTAGT

34501 GGCAAAGCCT CGCGCAAAAA AGAAAGCACA TCGTAGTCAT GCTCATGCAG ATAAAGGCAG GTAAGCTCCG GAACCACCAC AGAAAAAGAC ACCATTTTTC
CCGTTTCGGA GCGCGTTTTT TCTTTCGTGT AGCATCAGTA CGAGTACGTC TATTTCCGTC CATTCGAGGC CTTGGTGGTG TCTTTTTCTG TGGTAAAAAG

34601 TCTCAAACAT GTCTGCGGGT TTCTGCATAA ACACAAAATA AAATAACAAA AAAACATTTA AACATTAGAA GCCTGTCTTA CAACAGGAAA AACAACCCTT
AGAGTTTGTA CAGACGCCCA AAGACGTATT TGTGTTTTAT TTTATTGTTT TTTTGTAAAT TTGTAATCTT CGGACAGAAT GTTGTCCTTT TTGTTGGGAA

34701 ATAAGCATAA GACGGACTAC GGCCATGCCG GCGTGACCGT AAAAAAACTG GTCACCGTGA TTAAAAAGCA CCACCGACAG CTCCTCGGTC ATGTCCGGAG
TATTCGTATT CTGCCTGATG CCGGTACGGC CGCACTGGCA TTTTTTTGAC CAGTGGCACT AATTTTTCGT GGTGGCTGTC GAGGAGCCAG TACAGGCCTC

34801 TCATAATGTA AGACTCGGTA AACACATCAG GTTGATTCAC ATCGGTCAGT GCTAAAAAGC GACCGAAATA GCCCGGGGGA ATACATACCC GCAGGCGTAG
AGTATTACAT TCTGAGCCAT TTGTGTAGTC CAACTAAGTG TAGCCAGTCA CGATTTTTCG CTGGCTTTAT CGGGCCCCCT TATGTATGGG CGTCCGCATC

34901 AGACAACATT ACAGCCCCCA TAGGAGGTAT AACAAAATTA ATAGGAGAGA AAAACACATA AACACCTGAA AAACCCTCCT GCCTAGGCAA AATAGCACCC
TCTGTTGTAA TGTCGGGGGT ATCCTCCATA TTGTTTTAAT TATCCTCTCT TTTTGTGTAT TTGTGGACTT TTTGGGAGGA CGGATCCGTT TTATCGTGGG

35001 TCCCGCTCCA GAACAACATA CAGCGCTTCC ACAGCGGCAG CCATAACAGT CAGCCTTACC AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC
AGGGCGAGGT CTTGTTGTAT GTCGCGAAGG TGTCGCCGTC GGTATTGTCA GTCGGAATGG TCATTTTTTC TTTTGGATAA TTTTTTTGTG GTGAGCTGTG

35101 CGCACCAGCT CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA GTATATATAG GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA
GCGTGGTCGA GTTAGTCAGT GTCACATTTT TTCCCGGTTC ACGTCTCGCT CATATATATC CTGATTTTTT ACTGCATTGC CAATTTCAGG TGTTTTTTGT

35201 CCCAGAAAAC CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC CACAACTTCC TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTCACTTCC
GGGTCTTTTG GCGTGCGCTT GGATGCGGGT CTTTGCTTTC GGTTTTTTGG GTGTTGAAGG AGTTTAGCAG TGAAGGCAAA AGGGTGCAAT GCAGTGAAGG

Figure 15V

pMRKAd5gag MER682

```
35301  CATTTTAAGA AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA AACCTACGTC ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC
       GTAAAATTCT TTTGATGTTA AGGGTTGTGT ATGTTCAATG AGGCGGGATT TTGGATGCAG TGGGCGGGGC AAGGGTGCGG GGCGCGGTGC AGTGTTTGAG
                                                                   PacI
                                                                          EcoRI
35401  CACCCCCTCA TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGTTAA TTAAGAATTC GGATCTGCGA CGCGAGGCTG GATGGCCTTC
       GTGGGGGAGT AATAGTATAA CCGAAGTTAG GTTTTATTCC ATATAATAAC TACTACAATT AATTCTTAAG CCTAGACGCT GCGCTCCGAC CTACCGGAAG
35501  CCCATTATGA TTCTTCTCGC TTCCGGCGGC ATCGGGATGC CCGCGTTGCA GGCCATGCTG TCCAGGCAGG TAGATGACGA CCATCAGGGA CAGCTTCAAG
       GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCGCAACGT CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC
35601  GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA
       CGGTCGTTTT CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG GTATCCGAGG CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT
35701  GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC
       CTCCACCGCT TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC CTTCGAGGGA GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG
35801  CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG
       GACAGGCGGA AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC
35901  TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC
       ACGTGCTTGG GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG
36001  CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT
       GTGACCATTG TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC CACCGGATTG ATGCCGATGT GATCTTCCTG TCATAAACCA
36101  ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA
       TAGACGCGAG ACGACTTCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT
36201  AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT
       TCGTCGTCTA ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA
36301  TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA
       AAACCAGTAC TCTAATAGTT TTTCCTAGAA GTGGATCTAG GAAAATTTAG TTAGATTTCA TATATACTCA TTTGAACCAG ACTGTCAATG GTTACGAATT
36401  TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC
       AGTCACTCCG TGGATAGAGT CGCTAGACAG ATAAAGCAAG TAGGTATCAA CGGACTGAGG GGCAGCACAT CTATTGATGC TATGCCCTCC CGAATGGTAG
36501  TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT
       ACCGGGGTCA CGACGTTACT ATGGCGCTCT GGGTGCGAGT GGCCGAGGTC TAAATAGTCG TTATTTGGTC GGTCGGCCTT CCCGGCTCGC GTCTTCACCA
36601  CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG
       GGACGTTGAA ATAGGCGGAG GTAGGTCAGA TAATTAACAA CGGCCCTTCG ATCTCATTCA TCAAGCGGTC AATTATCAAA CGCGTTGCAA CAACGGTAAC
36701  CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA
       GATGTCCGTA GCACCACAGT GCGAGCAGCA AACCATACCG AAGTAAGTCG AGGCCAAGGG TTGCTAGTTC CGCTCAATGT ACTAGGGGGT ACAACACGTT
                                 PvuI
36801  AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT
       TTTTCGCCAA TCGAGGAAGC CAGGAGGCTA GCAACAGTCT TCATTCAACC GGCGTCACAA TAGTGAGTAC CAATACCGTC GTGACGTATT AAGAGAATGA
36901  GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT
       CAGTACGGTA GGCATTCTAC GAAAAGACAC TGACCACTCA TGAGTTGGTT CAGTAAGACT CTTATCACAT ACGCCGCTGG CTCAACGAGA ACGGGCCGCA
```

figure 15W

pMRKAd5gag MER6B2

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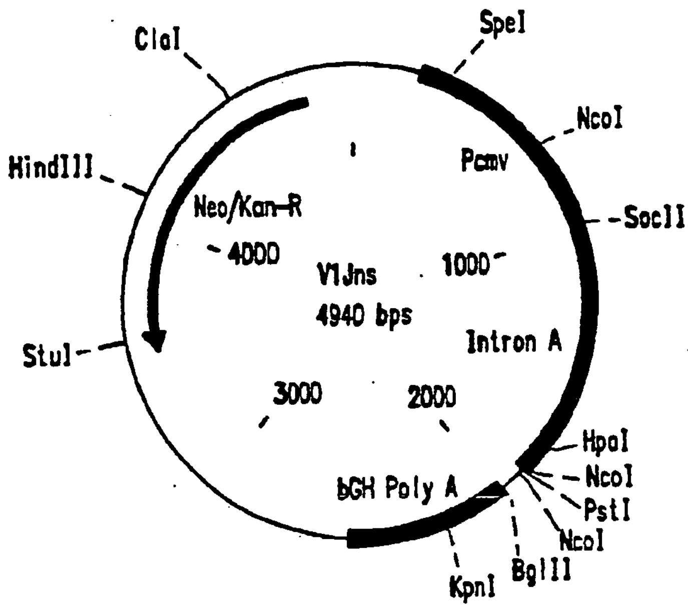
37001   CAACACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT
        GTTGTGCCCT ATTATGGCGC GGTGTATCGT CTTGAAATTT TCACGAGTAG TAACCTTTTG CAAGAAGCCC CGCTTTTGAG AGTTCCTAGA ATGGCGACAA
37101   GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT
        CTCTAGGTCA AGCTACATTG GGTGAGCACG TGGGTTGACT AGAAGTCGTA GAAAATGAAA GTGGTCGCAA AGACCCACTC GTTTTTGTCC TTCCGTTTTA
37201   GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA
        CGGCGTTTTT TCCCTTATTC CCGCTGTGCC TTTACAACTT ATGAGTATGA GAAGGAAAAA GTTATAATAA CTTCGTAAAT AGTCCCAATA ACAGAGTACT
37301   GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT
        CGCCTATGTA TAAACTTACA TAAATCTTTT TATTTGTTTA TCCCCAAGGC GCGTGTAAAG GGGCTTTTCA CGGTGGACTG CAGATTCTTT GGTAATAATA
                                                                              EcoRI
                                                                   BamHI
37401   CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC AAGAATTGGA TCCGAATTCT TAAT   (SEQ ID NO: 27)
        GTACTGTAAT TGGATATTTT TATCCGCATA GTGCTCCGGG AAAGCAGAAG TTCTTAACCT AGGCTTAAGA ATTA   (SEQ ID NO: 28)
```

Figure 15X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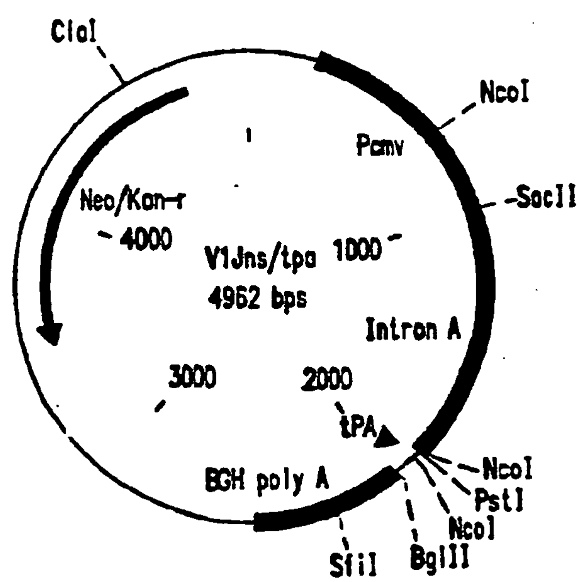
KpnI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NcoI
Pcmv
SacII
Neo/Kan-r
4000
V1Jns/tpa
1000
4962 bps
Intron A
3000
2000
tPA
NcoI
BGH poly A
PstI
NcoI
SfiI
BglII

FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA
BglII MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLy
1 10 20

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL
30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal
60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA
AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy
80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG
sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA
110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC
laPheThrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly
140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA
SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGl
160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC
euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis
220 230

CCCGACAAGTGGACTGTGCAGCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGl
240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC
yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL
270 280 290

FIGURE 17A

```
TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGACCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT
EuThrGluValIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis
                    300                           310

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA
GlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
   320                         330                          340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCACACCAATGATGTGAAGCAGCTGA
rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT
          350                          360                          370

CTGAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG
hrGluAlaValGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
                    380                           390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCCCT
GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe
   400                         410                          420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
uValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG
          430                          440                          450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAAGGTGGTGACCCTGACTGACACCACCAACCAG
luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln
                    460                           470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl
   480                         490                          500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG
aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG
          510                          520                          530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCCACAAGGGCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
luLysValTyrLeuAlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly
                    540                           550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
   560                         570                          580
```

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGGGGAGG
tAlaSerAspPheAsnLeuProProValValAlaLysGluIleValAlaSerCysAspLysCysGlnLeuLysGlyGluA
590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG
laMetHisGlyGlnValAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysValIleLeuVal
620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT
AlaValHisValAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG
uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA
670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC
laCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyValValAlaSerMetAsn
700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT
LysGluLeuLysLysIleIleGlyGlnValArgAspGlnAlaGluHisLeuLysThrAlaValGlnMetAlaValPheIl
720 730 740

CCACAACTTCAAGAGGAAGGGGGGCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleValAspIleIleAlaThrAspIleG
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
lnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGGTGCCCAG
LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaValValIleGlnAspAsnSerAspIleLysValValProAr
800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCCTCCAGGCAGGATGAGGACT
gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx
830 840 850

AAAGCCCGGGCAGATCT (SEQ ID NO: 3)
Xx BglII (SEQ ID NO: 4)

FIGURE 17C

```
GATCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGC
       MetAspAlaMetLysArgGlyLeuCysCysValLeuLeuLeuCysGlyAlaValPheValSerP
       -25           -20                            -10

CCAGCGAGATCTCCGCCCCCATCTCCCCCATTGAGACTGTGCCTGTGAAGCTGAAGCCTGGCATGGATGGC  (within SEQ ID NO: 7)
RoSerGluIleSerAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGly  (within SEQ ID NO: 8)
           -1 2                      10                           20
```

FIGURE 18

```
WT   - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT   -42
OPT  - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC
        M   G   G   K   W   S   K   R   S   V   P   G   W   S    -14

WT   - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT   -84
OPT  - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC
        T   V   R   E   R   M   R   R   A   E   P   A   A   D    -28

WT   - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA   -126
OPT  - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC
        R   V   R   R   T   E   P   A   A   V   G   V   G   A    -42

WT   - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC   -168
OPT  - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC
        V   S   R   D   L   E   K   H   G   A   I   T   S   S    -56

WT   - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA   -210
OPT  - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC
        N   T   A   A   T   N   A   D   C   A   W   L   E   A    -70

WT   - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA   -252
OPT  - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG
        Q   E   D   E   E   V   G   F   P   V   R   P   Q   V    -84

WT   - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC   -294
OPT  - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC
        P   L   R   P   M   T   Y   K   G   A   V   D   L   S    -98

WT   - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC   -336
OPT  - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC
        H   F   L   K   E   K   G   G   L   E   G   L   I   H    -112

WT   - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC   -378
OPT  - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC
        S   Q   K   R   Q   D   I   L   D   L   W   V   Y   H    -126

WT   - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG   -420
OPT  - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC
        T   Q   G   Y   F   P   D   W   Q   N   Y   T   P   G    -140
```

FIGURE 19A

```
WT    - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG    -462
        ||  ||  ||| ||  ||  ||  ||  ||| ||  ||  ||| ||| ||| |||
OPT   - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG
         P   G   I   R   F   P   L   T   F   G   W   C   F   K     -154

WT    - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA    -504
        ||  ||  ||  ||  ||| ||  ||  ||| ||  ||  ||| ||| ||  ||
OPT   - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG
         L   V   P   V   E   P   E   K   V   E   E   A   N   E     -168

WT    - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG    -546
        ||  ||| ||| ||| |||  ||  |  ||| ||  |||   | ||| ||  ||
OPT   - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC
         G   E   N   N   C   L   L   H   P   M   S   Q   H   G     -182

WT    - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC    -588
        ||  ||| ||| ||  ||| ||| ||  ||| | | ||| ||| ||| | | ||
OPT   - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC
         I   E   D   P   E   K   E   V   L   E   W   R   F   D     -196

WT    - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG    -630
          | ||| ||  ||  ||  ||  ||| ||| |||  |  ||| ||| ||  ||
OPT   - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC
         S   K   L   A   F   H   H   V   A   R   E   L   H   P     -210

WT    - GAG TAC TAC AAG GAC TGC TGA  (SEQ ID NO:30)                -651
        ||| ||| ||| ||| ||| ||| | |
OPT   - GAG TAC TAC AAG GAC TGC TAA  (contained within SEQ ID NO: 9)
         E   Y   Y   K   D   C       (SEQ ID NO: 10)               -216
```

FIGURE 19B

```
V1Jns/nef
               PstI                   BglII
CATGGGTCTTTTCTGCAGTCACCGTCCTTGAGATCTGCCACC ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC . . . .
                                            M   G   G   K   W   S   K   R   S   V   P

                                            SrfI    BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC (SEQ ID NO: 38)
           H   P   E   Y   Y   K   D   C   * (contained within SEQ ID NO:10)

V1Jns/nef(G2A.LLAA)
               PstI                   BglII
CATGGGTCTTTTCTGCAGTCACCGTCCTTGAGATCTGCCACC ATG GCC GGC AAG TGG TCC AAG AGG TCC GTG CCC . . . .
                                            M   A   G   K   W   S   K   R   S   V   P

                                            SrfI    BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC (SEQ ID NO: 39)
           H   P   E   Y   Y   K   D   C   *  (contained within SEQ ID NO:14)

V1Jns/tpanef & V1Jns/tpanef(LLAA)
               PstI
CATGGGTCTTTTCTGCAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG AAG AGA GGG CTC TGC TGT GTG
                                             M   D   A   M   K   R   G   L   C   C   V

                                                BglII
CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ATC TCC TCC AAG AGG TCC GTG CCC . . . .
 L   L   L   C   G   A   V   F   V   S   P   S   E   I   S   S   K   R   S   V   P

                                            SrfI    BglII
. . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGATCTGCTGTGCCTTCTAGTTGCCAGC (SEQ ID NO: 40)
           H   P   E   Y   Y   K   D   C   *  (contained withon SEQ ID NO: 16 )
```

FIGURE 20

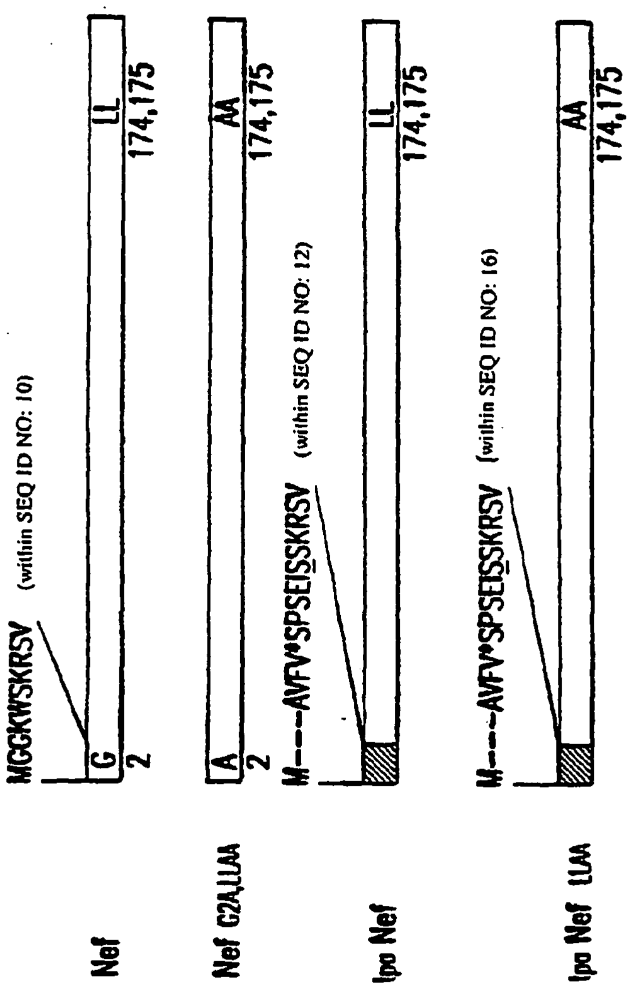

Nef
MGGKWSKRSV (within SEQ ID NO: 10)
G
2
LL
174,175
Nef G2A,LLAA
A
2
AA
174,175
lpo Nef
M----AVFV*SPSEISSKRSV (within SEQ ID NO: 12)
LL
174,175
lpo Nef LLAA
M----AVFV*SPSEISSKRSV (within SEQ ID NO: 16)
AA
174,175

FIGURE 21

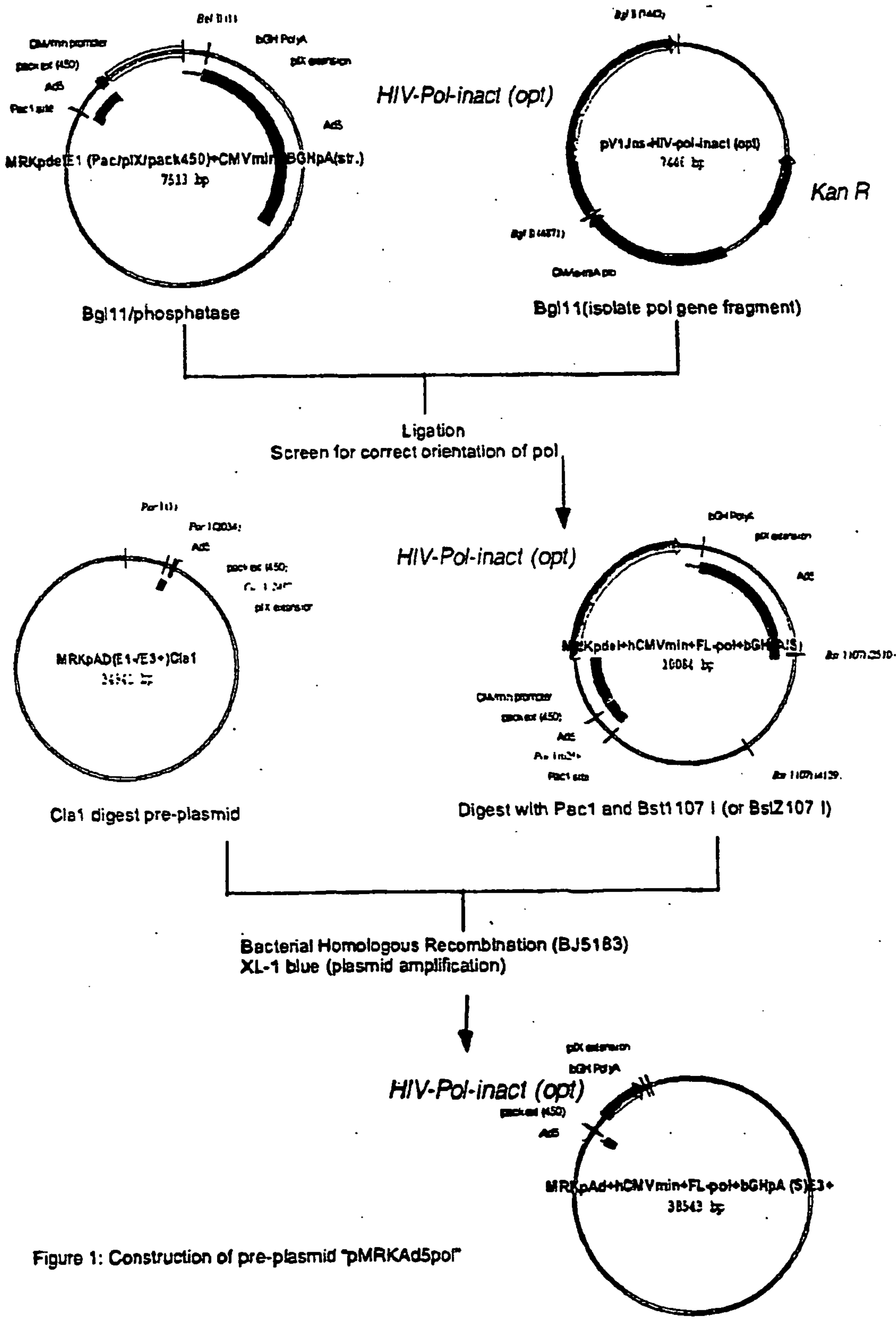


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

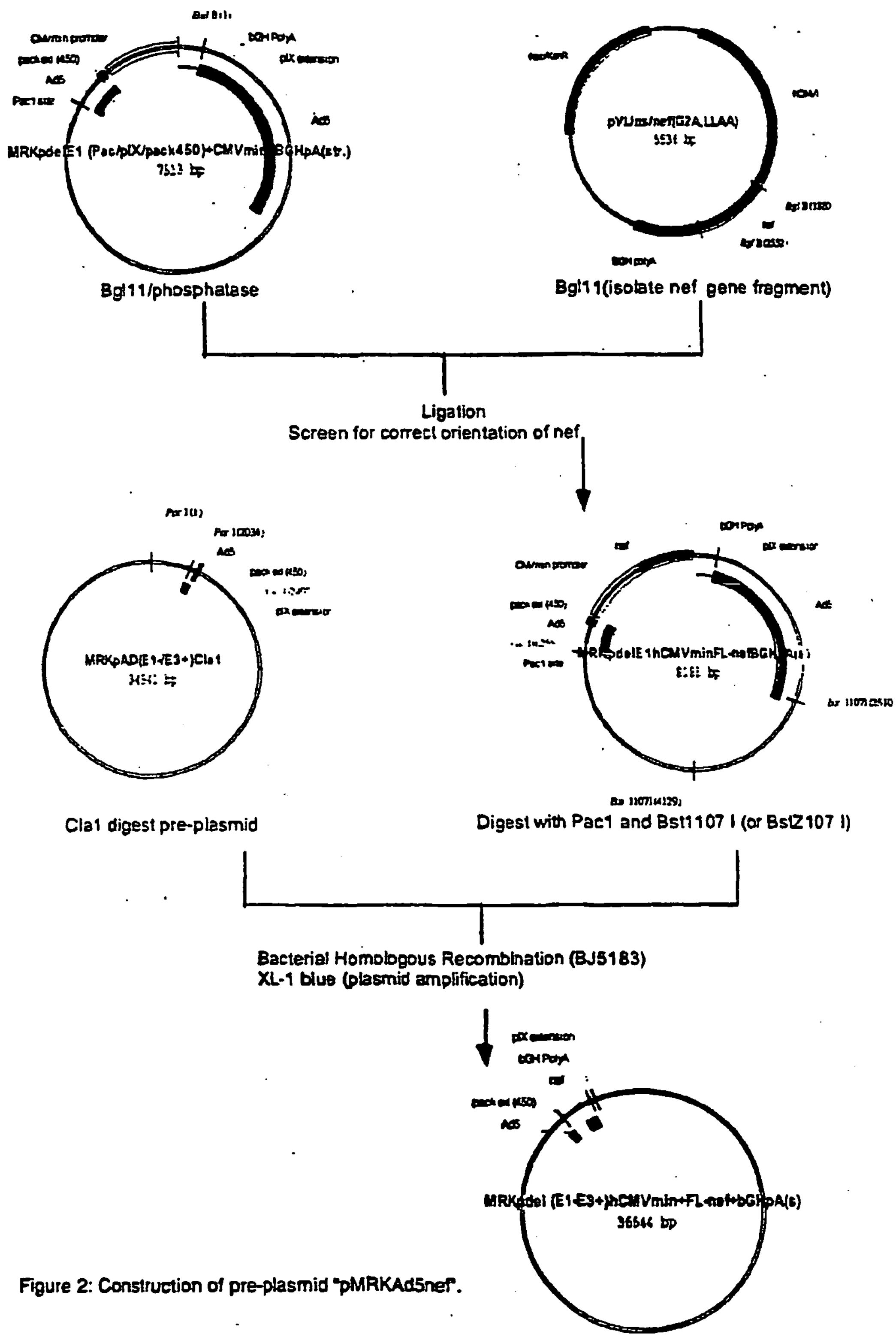
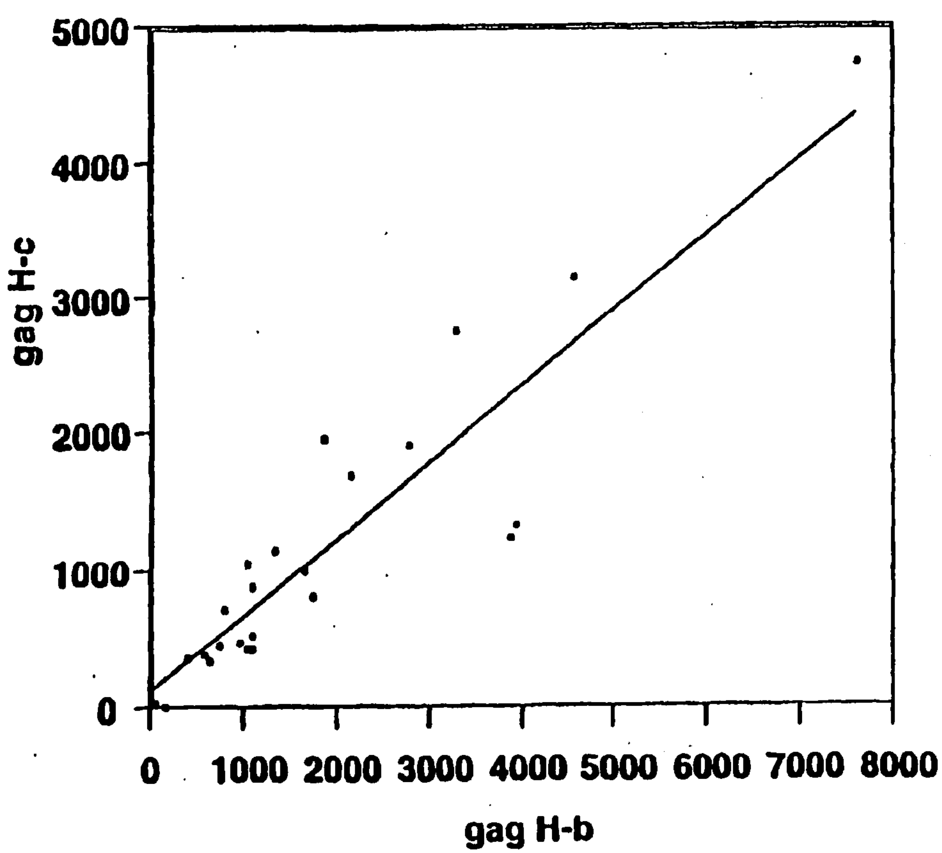


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

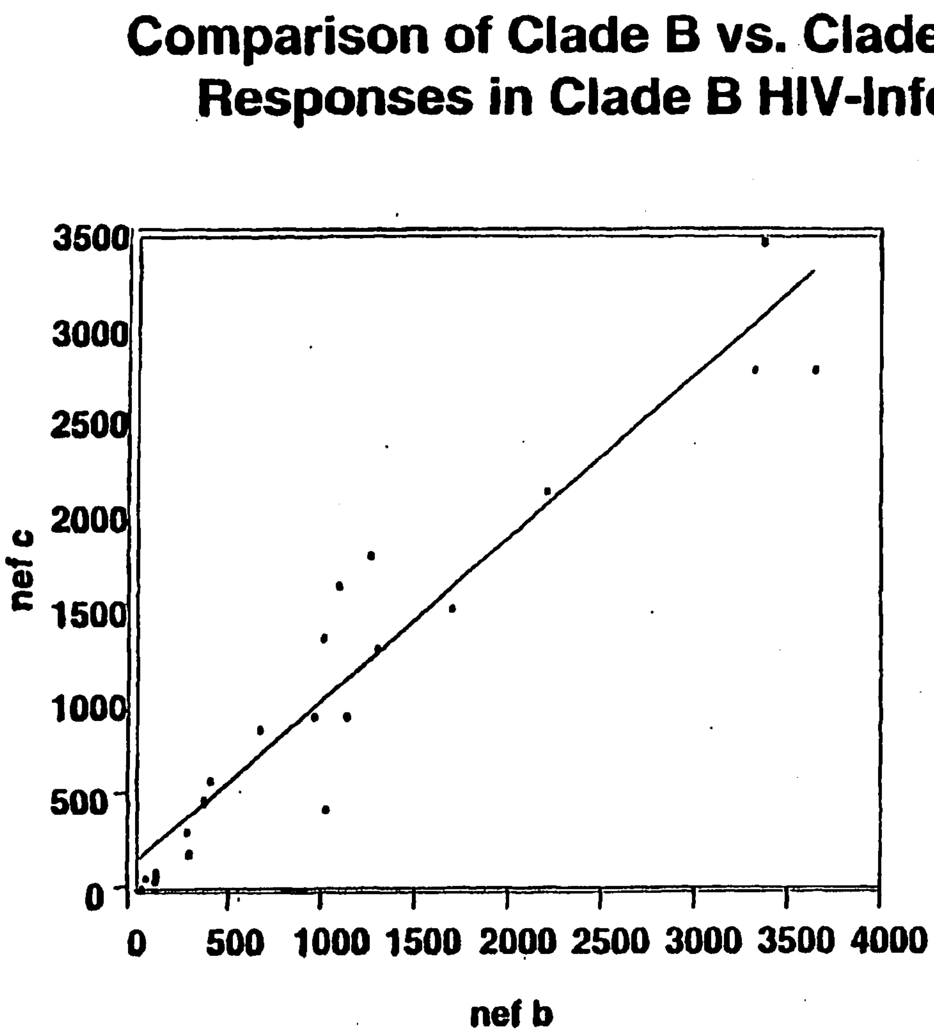


Linear Fit

gag H-c = 111.603 + 0.55866 gag H-b

Summary of Fit

RSquare	0.816775
RSquare Adj	0.80914
Root Mean Square Error	474.9639
Mean of Response	1158.115
Observations (or Sum Wgts)	26



nef c = 131.132 + 0.8646 nef b

Summary of Fit	
RSquare	0.91685
RSquare Adj	0.91289
Root Mean Square Error	289.7718
Mean of Response	1096.435
Observations (or Sum Wgts)	23

FIGURE 25

MRKAd5pol MER1062
(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

```
1   CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
    GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
    CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
    ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
    CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
    CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA
    GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
    TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
    CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
    GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
    CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
    ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
    ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
    ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
    GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
    TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
    TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
    GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
    GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT
```

Figure 26A

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
GGTAGTTCTT CTTCCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG ACACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26B

```
1901  GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG
      CCCCGGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC

1951  TGGATGGGCT ATGAGCTGCA CCCCGACAAG TGGACTGTGC AGCCCATTGT
      ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA

2001  GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG
      CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC

2051  GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
      CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC

2101  CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT
      GACACGTTCG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA

2151  GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG
      CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC

2201  AGCCTGTGCA TGGGGTGTAC TATGACCCCT CCAAGGACCT GATTGCTGAG
      TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC

2251  ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC
      TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTTAGA TGGTCCTCGG

2301  CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA
      GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCCGGGTGT

2351  CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
      GGTTACTACA CTTCGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC

2401  TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA
      AGGTAACACT AGACCCCGTT CTGGGGGTTC AAGTTCGACG GGTAGGTCTT

2451  GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC
      CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG

2501  CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG
      GACTCACCCT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC

2551  CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC
      GACCTCTTCC TCGGGTAACA CCCCCGACTC TGGAAGATAC ACCGACCCCG

2601  TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG
      ACGGTTGTCC CTCTGGTTCG ACCCGTTCCG ACCGATACAC TGGTTGTCCC

2651  GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
      CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG

2701  CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT
      GAGGTCCGGT AGATGGACCG GGAGGTCCTG AGACCGGACC TCCACTTGTA

2751  TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC
      ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG
```

Figure 26C

2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA
CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC
ACTCGTCCAC CTGTTCGACC ACAGACGACC GTAGTCCTTC CACGACAAGG

2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG

3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA
ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCCT

3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG
CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101 GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG
CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA
CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAACT

3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC
CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301 TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT
AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG
GTTCGTCCTC AAACCGTAGG GGATGTTGGG GGTCAGGGTC CCCCACCACC

3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG
GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC

3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACTT
CGACTCGTGG ACTTCTGTCG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG
GTTCTCCTTC CCCCCGTAGC CCCCGATGAG GCGACCCCTC TCCTAACACC

3551 ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
TGTAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG

3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG
TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCCT TGGGGGACAC

3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC
CTTCCCGGGA CGGTTCGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG

3701 AGGACAACTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC
TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

Figure 26 D

```
3801  GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
      CCTACTCCTG ATTTCGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG

3851  CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
      GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACTGGGA CCTTCCACGG

3901  ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT
      TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951  GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
      CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC

4001  GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
      CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051  ATGGCCGATC GGCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
      TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCCAC

4101  GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC
      CCTTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151  AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA
      TCGTCGGCGG CGGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201  GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
      CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251  GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAAACTCTAC
      CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301  TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT
      ATGGAACTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351  CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
      GGCGGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401  TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC
      AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451  CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
      GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501  GGGAACTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT
      CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCGTCCAA

4551  TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA
      AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601  AAAACCAGAC TCTGTTTGGA TTTGGATCAA GCAAGTGTCT TGCTGTCTTT
      TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651  ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTCG
      TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCCAGC
```

Figure 26E

```
4751  GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCACT
      CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA

4801  GCAGAGCTTC ATGCTGCGGG GTGGTGTTGT AGATGATCCA GTCGTAGCAG
      CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851  GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC
      CTCGCGACCC GCACCACGGA TTTTTACAGA AAGTCATCGT TCGACTAACG

4901  CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTTA AGCTGGGATG
      GTCCCCGTCC GGGAACCACA TTCACAAATG TTTCGCCAAT TCGACCCTAC

4951  GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
      CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001  GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC
      CGATACAAGG GTCGGTATAG GGAGGCCCCT AAGTACAACA CGTCTTGGTG

5051  CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG
      GTCGTGTCAC ATAGGCCACG TGAACCCTTT AAACAGTACA TCGAATCTTC

5101  GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
      CTTTACGCAC CTTCTTGAAC CTCTGCGGGA ACACTGGAGG TTCTAAAAGG

5151  ATGCATTCGT CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
      TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCGCC GCCGGACCCG

5201  GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT
      CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251  CGTCATAGGC CATTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT
      GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCCACGG TCTGACGCCA

5301  ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT
      TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351  TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TGCGGGGCGA
      AAGGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT

5401  TGAAGAAAAC GGTTTCCGGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
      ACTTCTTTTG CCAAAGGCCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC

5451  TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCGT AAATCACACC
      AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG

5501  TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
      ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551  TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
      ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601  CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG
      GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC
```

Figure 26F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC
AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG

5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC

5801 CGGCTTTCGC TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA

5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG
GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC

5901 TGAAGGGGTG CGCTCCGGGC TGCGCGCTGG CCAGGGTGCG CTTGAGGCTG
ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC

5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG
CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC

6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA

6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA
ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT

6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCTCAT

6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG
CCGTAGGCGC GGCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC

6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCCC ATGCTTTTTG
ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC

6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC
TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG

6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTCGA
CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT

6351 GCGGTGTTCC GCGGTCCTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA
CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT

6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCACCC TCCCCATCGC

6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT
CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACACT TCTGTGTACA

6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC

6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC
ACTGGCCCAC AAGGACTTCC CCCCGATATT TTCCCCCACC CCCGCGCAAG

Figure 26G

```
6651  AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT
      TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTC TAACAGTCAA

6701  TCCAAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT
      AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA

6751  GAGGGTGGCC GCATCCATCT GGTCAGAAAA GACAATCTTT TTGTTGTCAA
      CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT

6801  GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG
      CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC

6851  GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT
      CTCGCGTCCC AAACCAAAAA CAGCGCTAGC CGCGCGAGGA ACCGGCGCTA

6901  GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTCG GGAAAGACGG
      CAAATCGACG TGCATAAGCG CGCGTTGCGT GGCGGTAAGC CCTTTCTGCC

6951  TGGTGCGCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG
      ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC

7001  GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT
      CACTGTTCCA GTTGCGACCA CCGATGGAGA GGCGCATCCG CGAGCAACCA

7051  CCAGCAGAGG CGGCCGCCCT TGCGCGAGCA GAATGGCGGT AGGGGGTCTA
      GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCCAGAT

7101  GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC
      CGACGCAGAG CAGGCCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTCG

7151  AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG
      TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201  CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC
      GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCCTG

7251  CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTCG
      GGGTACCGTA CCCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC

7301  TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT
      ATTTGCATCT CCCCGAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA

7351  TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCG TGCGAGGGAG
      AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGCTCCCTC

7401  CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG
      GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC

7451  ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG
      TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501  GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG
      CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC
```

Figure 26 H

7601 TCTAGGGCGC AGTAGTCCAG GGTTTCCTTG ATGATGTCAT ACTTATCCTG
AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC

7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAAACTCT TCGCGGTCTT
AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA

7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA

7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG

7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG
CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC

7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
GTTTCCACAG GGACTGGTAC TGAAACTCCA TGACCATAAA CTTCAGTCAC

7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTTGGA
AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT

7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC

8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT

8051 CGGTTGTTAA TTACCTGGGC GGCGAGCACG ATCTCGTCAA AGCCGTTGAT
GCCAACAATT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA

8101 GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAACTACC

8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
TTCCGTTAAA AAATTCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG

8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT

8251 TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTCC

8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC

8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCG CGGCTAGGTC
CATTCGCCCA GAACAAGGGT CGCCAGGGTA GGTTCCAAGC GCCGATCCAG

8401 TCGCGCGGCA GTCACTAGAG GCTCATCTCC GCCGAACTTC ATGACCAGCA
AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT

8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
ACTTCCCGTG CTCGACGAAG GGTTTCCGGG GGTAGGTTCA TATCCAGAGA

Figure 26I

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTAA
CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG
TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT
CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCTTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCGGCTGC TTGTCCTTGA
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA
GCTCGGGTTT CAGGTCTACA GGCGCGCGCC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

9101 GGCGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG
CCGCCCGCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG
ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GGCGGCCCTC

9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG
TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCCTGAATC
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAACTA GAGGACTTAG

9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCG GTGAGCTTGA ACCTGAAAGA
ACCGCGGAGA CGCACTTCTG CTGCCCGGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGCC TGGCGCAAAA
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT

9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9501 GGCGGCGAGG TCGTTGGAAA TGCGGGCCAT GAGCTGCGAG AAGGCGTTGA
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA
GCCCGCGCGT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCG
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
AACTATAGGG GGTTCCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGCGAAGTTG AAAAACTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
CCGCTTCAAC TTTTTGACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC
AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA
TTTGGAGAGC TCTTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG
CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GGCGGATGGT
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTAGTAG
GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC

10501 TCTTGCATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTCC
AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG

10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT
ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAAA CCGGCATCCA

10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA
CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT

10651 AGCAGGGCTA GGTCGGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC
TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG

10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CGGTGGTATG
GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC

10751 CGCCCGTGTT GATGGTGTAA GTGCAGTTGG CCATAACGGA CCAGTTAACG
GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC

10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC
CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG

10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC
GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG

10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG
GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCCAC

10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA
CGGCCCCGAG GCCCCCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT

11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG
CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC

11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC
CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG

11101 ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA
TACCAGCCCT GCGAGACCGG CCAGTCCGCG CGCGTTAGCA ACTGCGAGAT

11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG
CTGGCACGTT TTCCTCTCGG ACATTCGCCC GTGAGAAGGC ACCAGACCAC

11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA
CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT

11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA
AGGCCGGCAG GCGGCACTAG GTACGCCAAT GGCGGGCGCA CAGCTTGGGT

11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG
CCACACGCTG CAGTCTGTTG CCCCCTCACG AGGAAAACCG AAGGAAGGTC

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATTA AGTGGCTCGC TCCCTGTAGC
TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGACTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTC

11551 ACCCCGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTTTGCTT
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCCAGATG CATCCGGTGC TGCGGCAGAT GCGCCCCCCT CCTCAGCAGC
AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCG

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCTC CCCTCCTCCT
CCGTTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG
AATGCTTGGG GGCGCCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GGCGCGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTCC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT
CACGTCGACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG
AGGTGCGTCC CGCGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCCG ACCTGGTAAC CGCATACGAG CAGACGGTGA
GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT
TGGTCCTCTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT
CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT
TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CGCGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTCAG GGATGCGCTG
AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG
GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCGC
ACCGGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCCATA GACAAGGAGG TAAAGATCGA
TTCTATATGG TATGGGGAAT GCAAGGGTAT CTGTTCCTCC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC
CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG
ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT
GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTTCCCGGGA

12651 GGCTGGCACG GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG
CCGACCGTGC CCGTCGCCGC TATCTCTCCG GCTCAGGATG AAACTGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG
CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG
CGGCCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT
GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG
TGATTCGCCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC

12901 CGGTGCGGGC GGCGCTGCAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC
GCCACGCCCG CCGCGACGTC TCGGTCGGCA GGCCGGAATT GAGGTGCCTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC
CTGACCGCGG TCCAGTACCT GGCGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG
ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG
TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCG ACGAGGCCGG
TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA
GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTCGCCGT

13201 ACGTGCAGAC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG
TGCACGTCTG GTTGGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

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13301  ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG
       TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGCACGGC GCCCCTGTCC

13351  AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA
       TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401  CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG
       GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451  TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAACTTGC
       ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTTGAACG

13501  AGGGGCTGTG GGGGGTGCGG GCTCCCACAG GCGACCGCGC GACCGTGTCT
       TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA

13551  AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT
       TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601  CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA
       GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651  CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC
       GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701  CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG
       GTCCTCTAAT GTTCACAGTC GGCGCGCGAC CCCGTCCTCC TGTGCCCGTC

13751  CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC
       GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801  CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG
       GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC

13851  CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT
       GTCGTCTCGC ACTCGGAATT GGACTACGCG CTGCCCCATT GCGGGTCGCA

13901  GGCGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA
       CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951  ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCATCG CGCGGCCGCC
       TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001  GTGAACCCCG AGTATTTCAC CAATGCCATC TTGAACCCGC ACTGGCTACC
       CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051  GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCGAG GGTAACGATG
       CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101  GATTCCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG
       CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAAGGGG CGTTGGCGTC

14151  ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA
       TGGGACGATC TCAACGTTGT CGCGCTCGTC CGTCTCCGCC GCGACGCTTT
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Figure 26 O

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14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC
      GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GGCGAGGAGG AGTACCTAAA
      TCGTGAGCGT GGTGGGCGGG CGCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTC
      GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG
      GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG
      ATGCGCGTCC TCGTGTCCCT GCACGGTCCG GGCGCGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG
      AGTTTCCGTG CTGGCAGTCG CCCCAGACCA CACCCTCCTG CTACTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG
      GTCTGCTGTC GTCGCAGGAC CTAAACCCTC CCTCACCGTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAAAAA AAAAGCATGA
      GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTTCTT
      ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCCCT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCCTCCT
      CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG
      GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTCACCGCC GCCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC
      AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC
      ACGCCGGATG GCCCCCCTCT TTGTCGTAGG CAATGAGACT CAACCGTGGG

14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT
      GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA
      CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001 TTCAAAACAA TGACTACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT
      AAGTTTTGTT ACTGATGTCG GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTTGACGACC GGTCGCACTG GGCGGCGAC CTGAAAACCA TCCTGCATAC
      GAACTGCTGG CCAGCGTGAC CCCGCCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC
      GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCCGCG
```

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA
CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT
ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG
CTGGTATCTG GAATACTTGT TGCGCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC
CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAAC GACGGTCCTA

15451 GCGGGGTGGA CTTCACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA
TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAC ATTCCCGCAC TGTTGGATGT GGACGCCTAC CAGGCGAGCT
CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GGCGGGGGTG GCGCAGGCGG CAGCAACAGC
ACTTTCTACT GTGGCTTGTC CCGCCCCCAC CGCGTCCGCC GTCGTTGTCG

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA
TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC
GTGCCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACCGGTGAT
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA
GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCGT

15901 ATGACAGCAC CTTCACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC
TACTGTCGTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GGCGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA
CCGCTGGGAG TCTGGCCTTA GGCGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26Q

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16151  GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT
       CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201  TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCCACC
       AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251  ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT
       TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301  ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG
       TGGCGACGCG TTGTCGTAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC

16351  CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG
       GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401  CCGCGCGTCC TATCGAGCCG CACTTTTTGA GCAAGCATGT CCATCCTTAT
       GGCGCGCAGG ATAGCTCGGC GTGAAAAACT CGTTCGTACA GGTAGGAATA

16451  ATCGCCCAGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT
       TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA

16501  TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG
       AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC

16551  CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC
       GTGATGGCGC GCGGGACCCC GCGCGTGTTT GCGCCGGCGT GACCCGCGTG

16601  CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA
       GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT

16651  CGCCCACGCC GCCACCAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG
       GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC

16701  GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT
       CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751  AGCACGTCGC CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG
       TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCCGCC

16801  CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGGC GGCCATGCGG
       GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC

16851  GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG
       CGGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GGTCCAGGTC

16901  GCGACGAGCG GCCGCCGCAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG
       CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951  GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC
       CAGCGTCCCC GTTGCACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG

17001  GTGCCCGTGC GCACCCGCCC CCCGCGCAAC TAGATTGCAA GAAAAAACTA
       CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT
```

Figure 26R

17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG
GATACAGGTT CGCGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCCCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA
CTCTAGATAC CGGGGGGCTT CTTCCTTCTC GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG
CGATTTCGCC CAGTTTTTCT TTTTCTTTCT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG
TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTCGAC GCGTAAAACG TGTTTTGCGA CCCGGCACCA CCGTAGTCTT
TTTCCAGCTG CGCATTTTGC ACAAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCGGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG
ATGCGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG
ACATGCCGCT GCTCCTGGAC GAACTCGTCC GGTTGCTCGC GGAGCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA
AAACGGATGC CTTTCGCCGT ATTCCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC
CCCGTTGGGT TGTGGATCGG ATTTCGGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT
GGCGCGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTCGC GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA
CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC
TCTACAGAAC CTTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG
CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTCAGA TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA
CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GGCGGTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCGGTTGC CTCAGCGGTG GCGGATGCCG
CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CGGTGCAGGC GGTCGCTGCG GCCGCGTCCA AGACCTCTAC GGAGGTGCAA
GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCGGCGCC CGCGCCGTTC
TGCCTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGGCAAG

17951 GAGGAAGTAC GGCGCCGCCA GCGCGCTACT GCCCGAATAT GCCCTACATC
CTCCTTCATG CCGCGGCGGT CGCGCGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG
TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC
AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCCAGC
CGCTTCCTCC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTCG

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG
TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA
GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGCGCACCAC
CCCCGTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCCT
GCCGCCGCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT
GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG
GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTTGTTC AACGTACACC

18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC
TTTTTAGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC
ATAAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACTGGCAA GATATCGGCA CCAGCAATAT
CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTCGTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT
CTCGCCACCG CGGAAGTCGA CCCCGAGCGA CACCTCGCCG TAATTTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA
AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT
CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC
CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA
TCCGTCACGT TTTATTCTAA TTGTCATTCG AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCGTGGCGA
CTCCTCGGAG GTGGCCGGCA CCTCTGTCAC AGAGGTCTCC CCGCACCGCT

Figure 26.T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT
TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC
GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG
CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC
CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCAGCG GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA
CGGCGGTCGC CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC
TTCGTGTGAC TTGTCGTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC
CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA
TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC
ACCGATGGGG AAGCTACTAC GGCGTCACCA GAATGTACGT GTAGAGCCCG

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCAGT TTGCCCGCGC
GTCCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG
GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG
GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT
AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT
GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT
AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCACTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA
CCGTGACGGA TGTTGCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG
TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAACTCAC
TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTTGAGTG

Figure 26 U

19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTC
AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG

20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT
TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTTG TCTTTAATTA

20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA
GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT

20101 CGGTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG
GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAAGAAC

20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC
ATTTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT
AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTCA

20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCCAGAC ACTCATATTT
CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301 CTTACATGCC CACTATTAAG GAAGGTAACT CACGAGAACT AATGGGCCAA
GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT

20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT
GTTAGATACG GGTTGTCCGG ATTAATGTAA CGAAAATCCC TGTTAAAATA

20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC
ACCAGATTAC ATAATGTTGT CGTGCCCATT ATACCCACAA GACCGCCCGG

20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG
TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCTGTC TTTGTGTCTC

20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT
GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA
AAGATACACC TTAGTCCGAC AACTGTCGAT ACTAGGTCTA CAATCTTAAT

20601 TTGAAAATCA TGGAACTGAA GATGAACTTC CAAATTACTG CTTTCCACTG
AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC

20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG
CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG
AGTCCTTTTA CCTACCCTTT TTCTACGATG TCTTAAAAGT CTATTTTTAC

20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC
TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG

20801 CTGTGGAGAA ATTTCCTGTA CTCCAACATA GCGCTGTATT TGCCCGACAA
GACACCTCTT TAAAGGACAT GAGGTTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 V

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20901  ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC
       TGCTGATGTA CTTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951  ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC
       TAATTGGAAC CTCGTGCGAC CAGGGAACTG ATATACCTGT TGCAGTTGGG

21001  ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG
       TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051  GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT
       CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101  GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA
       CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151  CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC
       GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201  TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC
       ATTCCCAACT GCCTCGGTCG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251  ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT
       TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301  TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA
       ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351  ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC
       TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTGCACGG GTATAGGTAG

21401  CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA
       GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451  GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT
       CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501  ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC
       TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551  TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA
       AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601  TGACCGCCTG CTTACCCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG
       ACTGGCGGAC GAATGGGGGT TGCTCAAACT TTAATTCGCG AGTCAACTGC

21651  GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCTG
       CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701  GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC
       CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751  AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA
       TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT
```

Figure 26 W

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC
CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA
GTGGTACGCG CTTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAACGCTA

22001 CGCACCCTTT GGCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCCACGCGC
TGAGTGTCTG GACCCGGTTT TGGAAGAGAT GCGGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCCAC CCTTCTTTAT
ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG
CAAAACAAAC TTCAGAAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA
GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA
GTTGTATTTC TTCGTTCGTT GTAGTTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGGTTG TGGGCCATAT
CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351 TTTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA
AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC
CGAGCGGACG CGGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGCATG

22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA
CTCGGGAAAC CGAAAAGACT GGTCGCTGAG TTCGTCCAAA TGGTCAAACT

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTTGACCGG

22701 CCAAACTCCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC
GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTTGGAATAA TGGCCCCATG

Figure 26 X

```
22801  CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCCGCAG
       GTCCTTGTCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851  CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTTGTCAC TTGAAAAACA
       GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAAACAGTG AACTTTTTGT

22901  TGTAAAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT
       ACATTTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAATA

22951  TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT
       AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001  TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG
       AATTTTTAGT TTCCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051  ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAAACTC AGGCACAACC
       TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101  ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC
       TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151  CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC
       GTTGCGCAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201  CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC
       GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251  ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT
       TGATAGTCGC GGCCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301  CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT
       GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351  TTGGTAGCTG CCTTCCCAAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC
       AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401  TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG
       AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451  ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT
       TATGTCGCGG ACGTATTTTC GGAACTAGAC GAATTTTCGG TGGACTCGGA

23501  TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAACTGATTG
       AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551  GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTGGAGAT
       CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601  CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG
       GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651  ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA
       TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT
```

Figure 26Y

```
23701 ATCACGTGCT CCTTATTTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC
      TAGTGCACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT
      CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGCGCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG
      GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG
      TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG
      GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC
      GAAGGTGAAC CAGTCCGTCA TCAAACTTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA
      TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT
      GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCCTCTT GCGTCCGCAT ACCACGCGCC
      GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGCGCGG

24151 ACTGGGTCGT CTTCATTCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC
      TGACCCAGCA GAAGTAAGTC GGCGGCGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTTGCTGAA ACCCACCATT TGTAGCGCCA
      TACGAACTAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG
      GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC
      GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTCG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA
      GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCCTC
      CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GGCGGCGACG GGGACGGGGA
      TAGGCGAAAA AACCCCCGCG GGCCCCTCCG CCGCCGCTGC CCCTGCCCCT

24501 CGACACGTCC TCCATGGTTG GGGGACGTCG CGCCGCACCG CGTCCGCGCT
      GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC
      GCCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC
      ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG
```

Figure 26Z

24701 CTACCACCTT CCCCGTCGAG GCACCCCCGC TTGAGGAGGA GGAAGTGATT
GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA

24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT
TAGCTCGTCC TGGGTCCAAA ACATTCGCTT CTGCTGCTCC TGGCGAGTCA

24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG
TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCGTCTC CGTTTGCTCC

24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA
TTGTTCAGCC CGCCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCCT

24901 GACGACGTGC TGTTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA
CTGCTGCACG ACAACTTCGT AGACGTCGCG GTCACGCGGT AATAGACGCT

24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCCT CGCCATAGCG GATGTCAGCC
GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG

25001 TTGCCTACGA ACGCCACCTA TTCTCACCGC GCGTACCCCC CAAACGCCAA
AACGGATGCT TGCGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT

25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCCGTATT
CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACTGCA
ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT

25151 AGATACCCCT ATCCTGCCGT GCCAACCGCA GCCGAGCGGA CAAGCAGCTG
TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTCGAC

25201 GCCTTGCGGC AGGGCGCTGT CATACCTGAT ATCGCCTCGC TCAACGAAGT
CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA

25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAACG
CGGTTTTTAG AAACTCCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACTCTGG AGTGTTGGTG
GAGACGTTGT CCTTTTGTCG CTTTTACTTT CAGTGAGACC TCACAACCAC

25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA
CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

25401 GGTCACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCCC AAGGTCATGA
CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT

25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG
CGTGTCAGTA CTCACTCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA
CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT

25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG
GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26 AA

```
25651  TGCATGCAGC GGTTCTTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA
       ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT

25701  AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA
       TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT

25751  TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC
       AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAAACGTG

25801  GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC
       CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851  GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT
       CGCGGCGCTG ATGCAGGCGC TGACGCAAAT GAATAAAGAT ACGATGTGGA

25901  GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC
       CCGTCTGCCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951  AAGGAGCTGC AGAAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC
       TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG

26001  CTTCAACGAG CGCTCCGTGG CCGCGCACCT GGCGGACATC ATTTTCCCCG
       GAAGTTGCTC GCGAGGCACC GGCGCGTGGA CCGCCTGTAG TAAAAGGGGC

26051  AACGCCTGCT TAAAACCCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA
       TTGCGGACGA ATTTTGGGAC GTTGTCCCAG ACGGTCTGAA GTGGTCAGTT

26101  AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT
       TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA

26151  GCCCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC
       CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201  GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC
       CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251  AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG
       TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301  TCTACTGGAG TGTCACTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC
       AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351  TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT
       ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401  GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTTGAA
       CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCCAACTT

26451  ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG
       TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501  AGGACTACCA CGCCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCG
       TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC
```

Figure 26 AB

```
26551  CCTAATGCGG AGCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCTTGG
       GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC

26601  CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG
       GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC

26651  GACGGGGGGT TTACTTGGAC CCCCAGTCCG GCGAGGAGCT CAACCCAATC
       CTGCCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG

26701  CCCCCGCCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA
       GGGGGCGGCG GCGTCGGGAT AGTCGTCGTC GGCGCCCGGG AACGAAGGGT

26751  GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG
       CCTACCGTGG GTTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC

26801  GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA
       CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT

26851  GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCG
       CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC

26901  AAGAGGTGTC AGACGAAACA CCGTCACCCT CGGTCGCATT CCCCTCGCCG
       TTCTCCACAG TCTGCTTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC

26951  GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC
       CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG

27001  TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA
       AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCCTGT

27051  CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA
       GGTGACCTTG GTCCCGGCCA TTCAGGTTCG TCGGCGGCGG CAATCGGGTT

27101  GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC
       CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG TGTTCTTGCG

27151  CATAGTTGCT TGCTTGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCGCC
       GTATCAACGA ACGAACGTTC TGACACCCCC GTTGTAGAGG AAGCGGGCGG

27201  GCTTTCTTCT CTACCATCAC GGCGTGGCCT TCCCCCGTAA CATCCTGCAT
       CGAAAGAAGA GATGGTAGTG CCGCACCGGA AGGGGGCATT GTAGGACGTA

27251  TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA
       ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT

27301  CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA
       GTCGTCGCCG GTGTGTCTTC GTTTCCGCTG GCCTATCGTT CTGAGACTGT

27351  AAGCCCAAGA AATCCACAGC GGCGGCAGCA GCAGGAGGAG GAGCGCTGCG
       TTCGGGTTCT TTAGGTGTCG CCGCCGTCGT CGTCCTCCTC CTCGCGACGC

27401  TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT
       AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCCTAAA

27451  TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG
       AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTTCTC
```

Figure 26. AC

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC
AGTGTTTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT
AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC
AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG

27701 GCCAGCACCT GTTGTCAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT
CGGTCGTGGA CAACAGTCGC GGTAATACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA
TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC
CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG
GGCCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTCC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC
GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC
CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG
GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTCGC CCGGGCAGGG TATAACTCAC
GCCCGCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC
GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GGCGCCGGCC
GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC
CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAACT CTGCAATTTA TTGAGGAGTT
AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC
ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC
GCCTAGTTAA ATAAGGATTG AAACTGCGCC ATTTCCTGAG CCGCCTGCCG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT
ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT
CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCGCTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCGAG GATCATATCG AGGGCCCGGC GCACGGCGTC
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT
GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG
TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG
GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT
AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT
TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT
CGCAGTGGCC GGCGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA

29051 AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT
TCTTTTGGGA ATCCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA
CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTTGG GGTTATTCTC TGTCTTGTGA TTCTCTTTAT TCTTATACTA
AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA
TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT
AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTACTCACCC TTGCGTCAGC CCACGGTACC ACCCAAAAGG
TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT
ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 2G AE

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29451  TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC
       AGCGGTGTTT TTGTTTTAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501  CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT
       GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551  AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT
       TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601  GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA
       CATGTACTCG TTTGTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651  ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG
       TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701  GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA
       CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751  GGAAAAGAAA ATGCCTTAAT TTACTAAGTT ACAAAGCTAA TGTCACCACT
       CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801  AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA
       TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT

29851  ATTAGAATAG GATTTAAACC CCCCGGTCAT TTCCTGCTCA ATACCATTCC
       TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901  CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA
       GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACTT

29951  GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC
       CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTCGT GGACAGGGCG

30001  GGATTTGTTC CAGTCCAACT ACAGCGACCC ACCCTAACAG AGATGACCAA
       CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051  CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC
       GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101  CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACTTGGG CATGTGGTGG
       GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151  TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG
       AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201  CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG
       GACGGATTTC GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251  TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC
       ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301  ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT
       TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA
```

Figure 26 AF

30401 TGCGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT
ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG
ACGAAATGCC TAAACAGTGG GAGTGCGAGT AGACGTCGGA GTAGTGACAC

30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA
CAGTAGCGGA AATAGGTCAC GTAACTGACC CAGACACACG CGAAACGTAT

30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA
AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT

30601 GAATTCTTTA ATTATGAAAT TTACTGTGAC TTTTCTGCTG ATTATTTGCA
CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

30651 CCCTATCTGC GTTTTGTTCC CCGACCTCCA AGCCTCAAAG ACATATATCA
GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG
ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTTCT
GCTAGAAAGG CTTCGGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG
CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAACT GTAACCGACC

30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT
TTGCGTTATC TACGGTACTT GGTGGGTTGA AAGGGGCGCG GGCGATACGA

30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC
AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

30951 GCCCACCTTC TCCCACCCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA
CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT

31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC
CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG

31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT
TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CGCGTACTTA

31101 CAAGAGCTCC AAGACATGGT TAACTTGCAC CAGTGCAAAA GGGGTATCTT
GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA

31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC
AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG

31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG
TGGCGGAATC GATGTTCAAC GGTTGGTTCG CAGTCTTTAA CCACCAGTAC

31251 GTGGGAGAAA AGCCCATTAC CATAACTCAG CACTCGGTAG AAACCGAAGG
CACCCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31351 AGACCCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT
TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC
GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG
CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACTGT GCCTTTTCTT ACTCCTCCCT TTGTATCCCC
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTTA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT
GAGAGAGACC TGCTCCGGCC GTTGGAATGG AGGGTTTTAC ATTGGTGACA

31851 GAGCCCACCT CTCAAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG
CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT
GTGGGGAGTG TCAATGGAGT CTTCGGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCGCTAAC
GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

32001 CGTGCACGAC TCCAAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT
GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGGCTA

32101 AGCAGTACCC TTACTATCAC TGCCTCACCC CCTCTAACTA CTGCCACTGG
TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC
ATCGAACCCG TAACTGAACT TTCTCGGGTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT
ATCCTGATTT CATGCCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26AH

32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC
TTGATTTCAA TGACCTCGGA ACCCAAAACT AAGTGTTCCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTTGTC TGCGGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT
GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTA TAAACTCAGC CCACAACTTG GATATTAACT
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT
CTCCAATTGG ATTCGTGACG GTTCCCCAAC TACAAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA
TGTGTTTAGG GGAGTTTTGT TTTTAACCGG TACCGGATCT TAAACTAAGT

32701 AACAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC
TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG

32751 AGGTGCCATT ACAGTAGGAA ACAAAAATAA TGATAAGCTA ACTTTGTGGA
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAACTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC
AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CCTTGTCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC
TTTCACGAGT AGAATAATAT TCTAAACTGC TTTTACCTCA CGATGATTTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG
ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCATTGTA ACAGTCAGTT

33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT
CAAATGAATT TGCCTCTGTT TTGATTTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTTCATG GGACTGGTCT GGCCACAACT ACATTAATGA AATATTTGCC
GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAATTACT TTATAAACGG

33301 ACATCCTCTT ACACTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG
TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGTTATGTTT CAACGTGTTT ATTTTTCAAT TGCAGAAAAT TTCAAGTCAT
ACAATACAAA GTTGCACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT
CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC
GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA
AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT
CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA
CCCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AAACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC
CGCGCGCTTA TTTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCCGCA GCATAAGGCG
TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCCT GATCTCACTT AAATCAGCAC
GGAACAGGAG GCCCGTGTCG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG
TCATTGACGT CGTGTCGTGG TGTTATAACA AGTTTTAGGG TGTCACGTTC

34001 GCGCTGTATC CAAAGCTCAT GGCGGGGACC ACAGAACCCA CGTGGCCATC
CGCGACATAG GTTTCGAGTA CCGCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG
TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC
TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCGC CGGCTATACA CTGCAGGGAA CCGGGACTGG
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT
CAGTACTATA GTTACAACCG TGTTGTGTCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTTGGG

34401 ATTCCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTTGG TCGTAGTGTC
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCCTGAAG CAAAACCAGG
TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC
ACGCCCGCAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGCTTCG GGTTCTATGT AAACTCCTTC ATGCGCCGCT GCCCTGATAA
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCGTTC
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG
AAAAAATAAG GTTTTCTAAT AGGTTTTGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GGCGTGGTCA AACTCTACAG CCAAAGAACA
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTTCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGCACAAT GGCTTCCAAA AGGCAAACGG
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC
GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26AK

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA
GGTGGAAGAG TTATATAGAG ATTCGTTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA
AACATTTTTA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA
TAGTACTAAC GTTTTTAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC
TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC
GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC
GCGGTCCTTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TGCGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA
GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTCG

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAAACA TTTAAACATT
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA
TCTTCGGACA GAATGTTGTC CTTTTTGTTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCATGTCC GGAGTCATAA TGTAAGACTC
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA
CCATTTGTGT AGTCCAACTA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTTGT GTATTTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA
ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

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36101  AAAGAAAACC TATTAAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA
       TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT

36151  GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA
       CAGTGTCACA TTTTTTCCCG GTTCACGTCT CGCTCATATA TATCCTGATT

36201  AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG
       TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC

36251  CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT
       GCTTGGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTTG AAGGAGTTTA

36301  CGTCACTTCC GTTTTCCCAC GTTACGTCAC TTCCCATTTT AAGAAAACTA
       GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT

36351  CAATTCCCAA CACATACAAG TTACTCCGCC CTAAAACCTA CGTCACCCGC
       GTTAAGGGTT GTGTATGTTC AATGAGGCGG GATTTTGGAT GCAGTGGGCG

36401  CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA
       GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT

                                                    PacI
                                                  --------
36451  TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA
       ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT

36501  ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC
       TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG

36551  TCGCTTCCGG CGGCATCGGG ATGCCCGCGT TGCAGGCCAT GCTGTCCAGG
       AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC

36601  CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG
       GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC

36651  GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
       CTTGGCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG

36701  CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG
       GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC

36751  ACAGGACTAT AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG
       TGTCCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC

36801  CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
       GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG

36851  CTTCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT
       GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA

36901  TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT
       AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA
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Figure 26 AM

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC
TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA
TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT

37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG
TGGTGGCGAC CATCGCCACC AAAAAAACAA ACGTTCGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG
GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT
AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT
CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG
GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA
ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG
GGTCGGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG
GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT
GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC
GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT
AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26AN

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37951  GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
       CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001  TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC
       ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051  CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT
       GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101  CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG
       GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151  TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
       ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201  CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
       GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTTCCCTT

38251  TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
       ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301  TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA
       ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351  ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
       TACATAAATC TTTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401  AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT
       TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451  AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA
       TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

                    PacI
                  ~~~~~~~
38501  TTCTTAATTT CTTAATTAA  (SEQ ID NO:32)
       AAGAATTAAA GAATTAATT  (SEQ ID NO:33)
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Figure 26 AO

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  1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
    GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

 51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
    CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
    ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
    CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
    CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA
    GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
    TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
    CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
    GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
    CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
    ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
    ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
    ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
    GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
    TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
    TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
    GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT
```

Figure 27A

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCGGCT
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
GGGTCTTCTC CGTCCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC
ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG
GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAACT GCGCCGCCCA CCCCATGTCC
ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG

Figure 27B

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
GAGGTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
TGTTCCTGAC GATTTCGGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCCTC CCCCGTGCCT TCCTTGACCC TGGAAGGTGC
GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA TCGCATTGTC
GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
ACTCATCCAC AGTAAGATAA GACCCCCCAC CCCACCCCGT CCTGTCGTTC

2101 GGGGAGGATT GGGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC
CCCCTCCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
ATACCGGCTA GCCGCGCGGC ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTTGT ATCTGTTTTG
CCCTTTCTTA TATATTCCAC CCCCAGAATA CATCAAAACA TAGACAAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG
GTCGTCGGCG GCGGCGGTAC TCGTGGTTGA GCAAACTACC TTCGTAACAC

2301 AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TGCGTCAGAA
TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCCTGCCC GCAAACTCTA
ACACTACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC
GATGGAACTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTCAGCCGC TGCAGCCACC GCCCGCGGGA TTGTGACTGA
AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG
GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAAACTGG

2601 CGGGAACTTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA
AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT
TTTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

Figure 27C

2751 TATTTAGGGG TTTTGCGCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGACTCTGGA
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTCC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTTG TAGATGATCC AGTCGTAGCA
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG
CCTCGCGACC CGCACCACGG ATTTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTTAGGTT
CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101 GGCTATGTTC CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA
CCGATACAAG GGTCGGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA
GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG
GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCGC CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTC CAGGATGAGA
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCCACGC TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG
AAAGGGTGCG AAACTCAAGT CTACCCCCCT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG
TACTTCTTTT GCCAAAGGCC CCATCCCCTC TAGTCGACCC TTCTTTCGTC

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCG TAAATCACAC
CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCATCC
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTC
GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

Figure 27 D

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3701  CCTGACCAAA TCCGCCAGAA GGCGCTCGCC GCCCAGCGAT AGCAGTTCTT
      GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA

3751  GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG
      CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801  CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC
      GAAAACTCGC AAACTGGTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851  CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
      GACGAGATGC CGTAGAGCTA GGTCGTATAG AGGAGCAAAG CGCCCAACCC

3901  GCGGCTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG
      CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951  TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG
      AGTACAGAAA GGTGCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001  GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT
      CACTTCCCCA CGCGAGGCCC GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051  GGTCCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
      CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101  GGTAGCATTT GACCATGGTG TCATAGTCCA GCCCCTCCGC GGCGTGGCCC
      CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151  TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
      AACCGCGCGT CGAACGGGAA CCTCCTCCGC GGCGTGCTCC CCGTCACGTC

4201  ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
      TGAAAACTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCTCA

4251  AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
      TCCGTAGGCG CGGCGTCCGG GGCGTCTGCC AGAGCGTAAG GTGCTCGGTC

4301  GTGAGCTCTG GCCGTTCGGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT
      CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351  GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA
      CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401  CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCCTCG
      GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACTCTCC GGACAGGAGC

4451  AGCGGTGTTC CGCGGTCCTC CTCGTATAGA AACTCGGACC ACTCTGAGAC
      TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501  AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
      TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTCACC CTCCCCATCG

4551  GGTCGTTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
      CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCCACAC TTCTGTGTAC

4601  TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTTTGTAGG TGTAGGCCAC
      AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG
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Figure 27E

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4701  CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT
      GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751  GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT
      CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801  TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGCG GTGATGCCTT
      AAGGTTTTTG CTCCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851  TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA
      ACTCCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901  AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT
      TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951  GGAGCGCAGG GTTTGGTTTT TGTCGCGATC GGCGCGCTCC TTGGCCGCGA
      CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGGCGCT

5001  TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG
      ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051  GTGGTGCGCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTTGTGCAG
      CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101  GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG
      CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151  TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT
      AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201  AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCAG
      TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTTCT GGGGCCCGTC

5251  CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT
      GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTC AGATCGCGGA

5301  GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA
      CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCCT

5351  CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC
      GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401  GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC
      CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451  TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA
      AAGGTGGCGC CTACGACCGC GCGTGCATTA GCATATCAAG CACGCTCCCT

5501  GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA
      CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551  GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT
      CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA
```

Figure 27F

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5651  GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC
      CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701  GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT
      CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751  GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAAACTC TTCGCGGTCT
      CAGGGAAAAA AAAGGTGTCG AGCGCCAACT CCTGTTTGAG AAGCGCCAGA

5801  TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC
      AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851  TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA
      ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901  CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC
      GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951  GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT
      CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA

6001  GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTTGG
      CAGCAGCGTA GGCGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051  AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC
      TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101  GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA
      CGCGCTCCGT ATTTCAACGC ACACTACGCC TTCCCAGGGC CGTGGAGCCT

6151  ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA
      TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201  TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG
      ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACTAC

6251  GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG
      CTTCCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301  CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA
      GGGCACGAGA CTTTCCCGGG TCAGACGTTC TACTCCCAAC CTTCGCTGCT

6351  ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG
      TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401  GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA
      CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451  GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTC GCGGCTAGGT
      CCATTCGCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501  CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACTT CATGACCAGC
      GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG
```

Figure 27G

```
6601  TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG
      ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC

6651  GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG
      CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACCGA TAACTACACC

6701  TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA
      ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT

6751  AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA
      TTTTGCACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT

6801  GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC
      CCAACTGGAC TGCTGGCGCG TGTTCCTTCG TCTCACCCTT AAACTCGGGG

6851  TCGCCTGGCG GGTTTGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG
      AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC

6901  ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC
      TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTGG TGGTGCGGCG

6951  GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTCGGAG CTTGATGACA
      CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT

7001  ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG
      TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC

7051  GTCAGGCGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC
      CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG

7101  GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGCG
      CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCGACCAA CCACCGCCGC

7151  TCGATGGCTT GCAAGAGGCC GCATCCCCGC GGCGCGACTA CGGTACCGCG
      AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC

7201  CGGCGGGCGG TGGGCCGCGG GGGTGTCCTT GGATGATGCA TCTAAAAGCG
      GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC

7251  GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA
      CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCCT GGGCGGCCCT

7301  GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC
      CTCCCCCGTC CCCGTGCAGC CGCGGCGCGC GCCCGTCCTC GACCACGACG

7351  GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTTGA TCTCCTGAAT
      CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA

7401  CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG
      GACCGCGGAG ACGCACTTCT GCTGCCCGGG CCACTCGAAC TTGGACTTTC

7451  AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAAA
      TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCCG GACCGCGTTT
```

Figure 27H

```
7551  CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG
      GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601  TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
      ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651  AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCGGCATC
      TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701  GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA
      CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751  AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG
      TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801  GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
      CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851  GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA
      CAACTATAGG GGGTTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901  CGGCGAAGTT GAAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
      GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951  TCCAGAAGAC GGATGAGCTC GGCGACAGTG TCGCGCACCT CGCGCTCAAA
      AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT

8001  GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT
      CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA

8051  CCCCTTCTTC TTCTTCTGGC GGCGGTGGGG GAGGGGGGAC ACGGCGGCGA
      GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCCTG TGCCGCCGCT

8101  CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
      GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151  GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA
      CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT

8201  GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG
      CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCCGAC

8251  CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT
      GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301  AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
      TCCATGAGGC GGCGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351  AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG
      TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401  AGCACCGTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA
      TCGTGGCACC GCCCGCCGTC GCCCGCCGCC AGCCCCAACA AAGACCGCCT
```

Figure 27I

8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG
AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC

8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA
AGCCGGTACG GGGTCCGAAG CAAAACTGTA GCCGCGTCCA GAAACATCAT

8601 GTCTTGCATG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTTGTC
CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG

8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG
GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC

8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG
ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC

8751 AAGCAGGGCT AGGTCGGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA
TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT

8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT
GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA

8851 GCGCCCGTGT TGATGGTGTA AGTGCAGTTG GCCATAACGG ACCAGTTAAC
CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG

8901 GGTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG
CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC

8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT
GGGAGCTCAG TTTATGCATC AGCAACGTTC AGGCGTGGTC CATGACCATA

9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT
GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA

9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT
CCGGCCCCGA GGCCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA

9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC
TCTACATGGA CCTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG

9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC
CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG

9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT
GTACCAGCCC TGCGAGACCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA

9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT
TCTGGCACGT TTTCCTCTCG GACATTCGCC CGTGAGAAGG CACCAGACCA

9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT
CCTATTTAAG CGTTCCCATA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA

9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTCGAACCC
TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9451 GGCGCGGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG
CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG CGCGCGTCGC

9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG
ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551 CCGGAGGGTT ATTTTCCAAG GGTTGAGTCG CGGGACCCCC GGTTCGAGTC
GGCCTCCCAA TAAAAGGTTC CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA
AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT
CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAAACGA

9701 TTTCCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCCC TCCTCAGCAG
AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCT CCCCTCCTCC
GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG
TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901 GGCGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG
CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTC

9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG
ACAAAGCGCT GGCGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GGCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT
AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCGGGATT AGTCCCGCGC
CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GGCGGCCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG
CGCGTGTGCA CCGCCGGCGG CTGGACCATT GGCGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAACTTTCA AAAAAGCTTT AACAACCACG TGCGTACGCT
TTGGTCCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG
ATTCGCGCGA CCTCGTTTTG GGTTTATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

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10401  GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA
       CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT

10451  TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG
       AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC

10501  GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCG
       CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC

10551  CAAGATATAC CATACCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG
       GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCCTC CATTTCTAGC

10601  AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC
       TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG

10651  CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG
       GACCCGCAAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC

10701  GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC
       CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCCGGG

10751  TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG
       ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC

10801  GGCGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG
       CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC

10851  GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG
       CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCGC

10901  GCGTGGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG
       CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC

10951  TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG
       ATGATTCGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC

11001  GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA
       CGCCACGCCC GCCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT

11051  CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC
       GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG

11101  CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG
       GACTGCGCAA GGCCGTCGTC GGCGTCCGGT TGGCCGAGAG GCGTTAAGAC

11151  GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC
       CTTCGCCACC AGGGCCGCGC GCGTTTGGGG TGCGTGCTCT TCCACGACCG

11201  GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG
       CTAGCATTTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC

11251  GCCTGGTCTA CGACGCGCTG CTTCAGCGCG TGGCTCGTTA CAACAGCGGC
       CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCCG
```

Figure 27L

11351 GGCGCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG
CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC

11401 CACTAAACGC CTTCCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG
GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTTGCACGG CGCCCCTGTC

11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGACTGAGAC
CTCCTGATGT GGTTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG

11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA
TGGCGTTTCA CTCCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT

11551 GTAGACAAGG CCTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAAACTTG
CATCTGTTCC GGACGTCTGG CATTTGGACT CGGTCCGAAA GTTTTTGAAC

11601 CAGGGGCTGT GGGGGGTGCG GGCTCCCACA GGCGACCGCG CGACCGTGTC
GTCCCCGACA CCCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG

11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT
ATCGAACGAC TGCGGGTTGA GCGCGGACAA CGACGACGAT TATCGCGGGA

11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACCTAGG TCACTTGCTG
AGTGCCTGTC ACCGTCGCAC AGGGCCCTGT GTATGGATCC AGTGAACGAC

11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT
TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA

11801 CCAGGAGATT ACAAGTGTCA GCCGCGCGCT GGGGCAGGAG GACACGGGCA
GGTCCTCTAA TGTTCACAGT CGGCGCGCGA CCCCGTCCTC CTGTGCCCGT

11851 GCCTGGAGGC AACCCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC
CGGACCTCCG TTGGGATTTG ATGGACGACT GGTTGGCCGC CGTCTTCTAG

11901 CCCTCGTTGC ACAGTTTAAA CAGCGAGGAG GAGCGCATTT TGCGCTACGT
GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA

11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCCAGCG
CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TGCGGGTCGC

12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA
ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATACGGAGT

12051 AACCGGCCGT TTATCAACCG CCTAATGGAC TACTTGCATC GCGCGGCCGC
TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG

12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC
GCACTTGGGG CTCATAAAGT GGTTACGGTA GAACTTGGGC GTGACCGATG

12151 CGCCCCCTGG TTTCTACACC GGGGGATTCG AGGTGCCCGA GGGTAACGAT
GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA

12201 GGATTCCTCT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA
CCTAAGGAGA CCCTGCTGTA TCTGCTGTCG CACAAAAGGG GCGTTGGCGT

Figure 27 M

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12301  AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
       TCCTTTCGAA GGCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351  CCGCGGTCAG ATGCTAGTAG CCCATTTCCA AGCTTGATAG GGTCTCTTAC
       GGCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401  CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA
       GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451  ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATTT
       TGTTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501  CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
       GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551  GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC
       CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601  GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
       CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCCTCCT GCTACTGAGC

12651  GCAGACGACA GCAGCGTCCT GGATTTGGGA GGGAGTGGCA ACCCGTTTGC
       CGTCTGCTGT CGTCGCAGGA CCTAAACCCT CCCTCACCGT TGGGCAAACG

12701  GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAAAAAAAA AAAAAGCATG
       CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751  ATGCAAAATA AAAAACTCAC CAAGGCCATG GCACCGAGCG TTGGTTTTCT
       TACGTTTTAT TTTTTGAGTG GTTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801  TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC
       ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851  TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
       AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTCACCGC CGCCGCGACC

12901  GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC
       CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951  CTGCGGCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC
       GACGCCGGAT GGCCCCCCTC TTTGTCGTAG GCAATGAGAC TCAACCGTGG

13001  CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
       GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTC AGTTGCCTAC

13051  TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
       ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101  ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
       TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151  TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
       AGAACTGCTG GCCAGCGTGA CCCCGCCGCT GGACTTTTGG TAGGACGTAT
```

Figure 27N

```
13251  CGGGTGATGG TGTCGCGCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA
       GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT

13301  ATACGAGTGG GTGGAGTTCA CGCTGCCCGA GGGCAACTAC TCCGAGACCA
       TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT

13351  TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG
       ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTCAC

13401  GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC
       CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACTGTG

13451  CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG
       GGCGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC

13501  GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA
       CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAGTAAAA CGACGGTCCT

13551  TGCGGGGTGG ACTTCACCCA CAGCCGCCTG AGCAACTTGT TGGGCATCCG
       ACGCCCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC

13601  CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG
       GTTCGCCGTT GGGAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC

13651  AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC
       TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG

13701  TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG
       AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC

13751  CAGTGGCAGC GGCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC
       GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG

13801  AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC
       TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG

13851  ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC
       TGTGCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG

13901  CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA
       GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT

13951  TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC
       AGTTTGGGGA CTGTCTCCTG TCGTTCTTTG CGTCAATGTT GGATTATTCG

14001  AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA
       TTACTGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT

14051  CGGCGACCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACTCCTG
       GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC

14101  ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTCGTTGCC AGACATGATG
       TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC
```

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAC
AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG GTCGGGGGTG

14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC
ATGGCGACGC GTTGTCGTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA
CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCCAG CAATAACACA GGCTGGGGCC TGCGCTTCCC AAGCAAGATG
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG
AAACCGCCCC GGTTCTTCGC GAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA
CGTGATGGCG CGCGGGACCC CGCGCGTGTT TGCGCCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCCACGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT
TGCGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCCCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG
CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG
ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA
CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001 GGCGACGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG
CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAATCACG ATACTGAGTC

15051 GGTCGCAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG
CCAGCGTCCC CGTTGCACAT AACCCACGCG CTGAGCCAAT CGCCGGACGC

Figure 27P

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15151  ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA
       TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201  GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC
       CGATACAGGT TCGCGTTTTA GTTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251  GGAGATCTAT GGCCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA
       CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGCTT

15301  AGCTAAAGCG GGTCAAAAAG AAAAAGAAAG ATGATGATGA TGAACTTGAC
       TCGATTTCGC CCAGTTTTTC TTTTTCTTTC TACTACTACT ACTTGAACTG

15351  GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG
       CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC

15401  GAAAGGTCGA CGCGTAAAAC GTGTTTTGCG ACCCGGCACC ACCGTAGTCT
       CTTTCCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451  TTACGCCCGG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG
       AATGCGGGCC ACTCGCGAGG TGGGCGTGGA TGTTCGCGCA CATACTACTC

15501  GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA
       CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTGCTCG CGGAGCCCCT

15551  GTTTGCCTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG
       CAAACGGATG CCTTTCGCCG TATTCCTGTA CGACCGCAAC GGCGACCTGC

15601  AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG
       TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651  CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCCTAAAGC GCGAGTCTGG
       GGGCGCGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCG CGCTCAGACC

15701  TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG
       ACTGAACCGT GGGTGGCACG TCGACTACCA TGGGTTCGCG GTCGCTGACC

15751  AAGATGTCTT GGAAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC
       TTCTACAGAA CCTTTTTTAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801  CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGACTGGGCG TGCAGACCGT
       GCGCACGCCG GTTAGTTCGT CCACCGCGGC CCTGACCCGC ACGTCTGGCA

15851  GGACGTTCAG ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG
       CCTGCAAGTC TATGGGTGAT GGTCATCGTG GTCATAACGG TGGCGGTGTC

15901  AGGGCATGGA GACACAAACG TCCCCGGTTG CCTCAGCGGT GGCGGATGCC
       TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCCTACGG

15951  GCGGTGCAGG CGGTCGCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA
       CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001  AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGGCGC CCGCGCCGTT
       TTGCCTGGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GGCGCGGCAA
```

Figure 27Q

16051 CGAGGAAGTA CGGCGCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT
CAGCGGCAGC GGTCGGGCAC GACCGGGGCT AAAGGCACGC GTCCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCCAG
GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA
TCCCCGTACC GGCCGGTGCC GGACTGCCCG CCGTACGCAG CACGCGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTCGCAT GCGCGGCGGT ATCCTGCCCC
GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA CGCGCCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA
AGGAATAAGG TGACTAGCGG CGCCGCTAAC CGCGGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAAAAACAA GTTGCATGTG
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTTGTT CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCCTGTAA
CTTTTTAGTT TTATTTTTCA GACCTGAGAG TGCGAGCGAA CCAGGACATT

16651 CTATTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCCGCGACA
GATAAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTTA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGA CCTGGCCAAC
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC
TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCACTAAAG CAAGGCCTGC CCACCACCCG
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGGC

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA
AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCGACCG CCGTTGTTGT AACCCGTCCT AGCCGCGCGT CCCTGCGCCG
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGGC

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC
GCGGCGGTCG CCAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC
TTTCGTGTGA CTTGTCGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GGCGCGCGGG CGAAAGGTTC

17501 ATGGCTACCC CTTCGATGAT GCCGCAGTGG TCTTACATGC ACATCTCGGG
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGCAG TTTGCCCGCG
GGTCCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCGG TCCCAGCGTT TGACGCTGCG
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAA TAAACCTAGA AGAAGAGGAC
TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

Figure 27S

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAACTCA
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTTTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTCGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAA

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT
AGTACGTCGA CCCTCTCAGG ATTTTTTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTCATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT
TGCCAAGTAT ACGTTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTTT
CATTTCGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCCAGA CACTCATATT
ACCATAACAT GTCACTTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTTGTAGAT TTGCAAGACA GAAACACAGA
GTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAACTGA AGATGAACTT CCAAATTACT GCTTTCCACT
TAACTTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAAACAG
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAT
CAGTCCTTTT ACCTACCCTT TTTCTACGAT GTCTTAAAAG TCTATTTTTA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

```
18951  AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC
       TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001  TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA
       ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT

19051  CATTAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC
       GTAATTGGAA CCTCGTGCGA CCAGGGAACT GATATACCTG TTGCAGTTGG

19101  CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG
       GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151  GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT
       CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201  TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA
       ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251  ACTTCAGGAA GGATGTTAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC
       TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301  CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC
       GATTCCCAAC TGCCTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG

19351  CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC
       GTGGAAGAAG GGGTACCGGG TGTTGTGGCG GAGGTGCGAA CTCCGGTACG

19401  TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC
       AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG

19451  AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT
       TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA

19501  CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA
       GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551  AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC
       TCTGATTCCT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601  TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC
       ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651  CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA
       GAAATTCTTC CACCGGTAAT GGAAACTGAG AAGACAGTCG ACCGGACCGT

19701  ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC
       TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTCGC GAGTCAACTG

19751  GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCCT
       CCCCTCCCAA TGTTGCAACG GGTCACATTG TACTGGTTTC TGACCAAGGA

19801  GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC
       CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG
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Figure 27 U

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19851  CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC
       GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901  ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT
       TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCCTGA TGGTTGTCCA

19951  GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC
       CCCGTAGGAT GTGGTTGTGT TGTTGAGACC TAAACAACCG ATGGAACGGG

20001  CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACTTCCC CTATCCGCTT
       GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051  ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA
       TATCCGTTCT GGCGTCAACT GTCGTAATGG GTCTTTTTCA AAGAAACGCT

20101  TCGCACCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG
       AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151  CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCCACGCG
       GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TGCGGTTGAG GCGGGTGCGC

20201  CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA
       GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGGAAGAAAT

20251  TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG
       ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GGCGTGGCGC

20301  GCGTCATCGA AACCGTGTAC CTGCGCACGC CCTTCTCGGC CGGCAACGCC
       CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGCGG

20351  ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC
       TGTTGTATTT CTTCGTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401  AGTGAGCAGG AACTGAAAGC CATTGTCAAA GATCTTGGTT GTGGGCCATA
       TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451  TTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA
       AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501  AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA
       TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551  CACTGGATGG CCTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT
       GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601  TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG
       ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651  AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCGACCGC
       TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701  TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC
       ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTTGAGCCG

20751  CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC
       GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG
```

Figure 27V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TGCGTCGCAA
GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA
GGTCCTTGTC GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTTGTCA CTTGAAAAAC
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA
TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG
AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC

21101 TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG
AAATTTTTAG TTTCCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC
CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA
GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GGCGCCGATA TCTTGAAGTC GCAGTTGGGG
GGTTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCGATAC ACAGGGTTGC AGCACTGGAA
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTCGGAGA
GTGATAGTCG CGGCCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCAGGCT TTGAGTTGCA
AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCGGTC TGGGCGTTAG
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC
CTATGTCGCG GACGTATTTT CGGAACTAGA CGAATTTTCG GTGGACTCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA
CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTCGGCCC CACCGGTTCT TCACGATCTT GGCCTTGCTA
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

```
21801  AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT
       TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA

21851  CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC
       GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTGCGCGT CGGGCACCCG

21901  TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG
       AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TGCGGACGTC

21951  GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT
       CTTAGCGGGG TAGTAGCAGT GTTTCCAGAA CAACGACCAC TTCCAGTCGA

22001  GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCATAC GGCCGCCAGA
       CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT

22051  GCTTCCACTT GGTCAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC
       CGAAGGTGAA CCAGTCCGTC ATCAAACTTC AAGCGGAAAT CTAGCAATAG

22101  CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC
       GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGGAAGAGGG

22151  ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT
       TGCGTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA

22201  TCCGCTTCGC TGGGCTCTTC CTCTTCCTCT TGCGTCCGCA TACCACGCGC
       AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCGCG

22251  CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC
       GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG

22301  CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCCACCAT TTGTAGCGCC
       GTACGAACTA ATCGTGGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG

22351  ACATCTTCTC TTTCTTCCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG
       TGTAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401  GCGCTCGGGC TTGGGAGAAG GGCGCTTCTT TTTCTTCTTG GGCGCAATGG
       CGCGAGCCCG AACCCTCTTC CCGCGAAGAA AAAGAAGAAC CCGCGTTACC

22451  CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC
       GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG

22501  AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT
       TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA

22551  CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG
       GTAGGCGAAA AAACCCCCGC GGGCCCCTCC GCCGCCGCTG CCCCTGCCCC

22601  ACGACACGTC CTCCATGGTT GGGGGACGTC GCGCCGCACC GCGTCCGCGC
       TGCTGTGCAG GAGGTACCAA CCCCCTGCAG CGCGGCGTGG CGCAGGCGCG

22651  TCGGGGGTGG TTTCGCGCTG CTCCTCTTCC CGACTGGCCA TTTCCTTCTC
       AGCCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG
```

Figure 27X

```
22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG
      GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCCG CTTGAGGAGG AGGAAGTGAT
      GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG
      ATAGCTCGTC CTGGGTCCAA AACATTCGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG
      ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTGCGTCT CCGTTTGCTC

22951 GAACAAGTCG GGCGGGGGGA CGAAAGGCAT GGCGACTACC TAGATGTGGG
      CTTGTTCAGC CCGCCCCCCT GCTTTCCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG
      TCTGCTGCAC GACAACTTCG TAGACGTCGC GGTCACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC
      TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA
      GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT
      TCTTTTGCCG TGTACGCTCG GGTTGGGCGC GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAAACTGC
      AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTTTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT
      TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTCGTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG
      CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC
      ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTTGGT
      CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG
      CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCACCCA CTTTGCCTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG
      TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG
      TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG
      CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC
```

Figure 27 Y

23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG
TTTGTAACGT GATGTGGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTC

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG
GCTTTTGGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC
GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT
ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG
GTTCCTCGAC GTCTTTGACG ATTTCGTTTT GAACTTCCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC
GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAAAACCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA
CTTGCGGACG AATTTTGGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACTTTAT CCTAGAGCGC TCAGGAATCT
TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCCTAGCG ACTTTGTGCC CATTAAGTAC
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG
GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTCGCAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT
TTGAGTGAGG CCCCGACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 27Z

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24601  GAGGACTACC ACGCCCACGA GATTAGGTTC TACGAAGACC AATCCCGCCC
       CTCCTGATGG TGCGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651  GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG
       CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC

24701  GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG
       CGGTTAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC

24751  GGACGGGGGG TTTACTTGGA CCCCCAGTCC GGCGAGGAGC TCAACCCAAT
       CCTGCCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA

24801  CCCCCCGCCG CCGCAGCCCT ATCAGCAGCA GCCGCGGGCC CTTGCTTCCC
       GGGGGGCGGC GGCGTCGGGA TAGTCGTCGT CGGCGCCCGG GAACGAAGGG

24851  AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA
       TCCTACCGTG GGTTTTTCTT CGACGTCGAC GGCGGCGGTG GGTGCCTGCT

24901  GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGGAC GAGGAGGAGG
       CCTCCTTATG ACCCTGTCAG TCCGTCTCCT CCAAAACCTG CTCCTCCTCC

24951  AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC
       TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001  GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTCGCAT TCCCCTCGCC
       CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051  GGCGCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC
       CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101  CTCAGGCGCC GCCGGCACTG CCCGTTCGCC GACCCAACCG TAGATGGGAC
       GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG

25151  ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA
       TGGTGACCTT GGTCCCGGCC ATTCAGGTTC GTCGGCGGCG GCAATCGGGT

25201  AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG
       TCTCGTTGTT GTCGCGGTTC CGATGGCGAG TACCGCGCCC GTGTTCTTGC

25251  CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTCGCCCGC
       GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301  CGCTTTCTTC TCTACCATCA CGGCGTGGCC TTCCCCCGTA ACATCCTGCA
       GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT

25351  TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA
       AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCCG TCGCCGTCGT

25401  ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC
       TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCCTATCGT TCTGAGACTG

25451  AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC
       TTTCGGGTTC TTTAGGTGTC GCCGCCGTCG TCGTCCTCCT CCTCGCGACG

25501  GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT
       CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCCTAA
```

Figure 27 AA

```
25551  TTTCCCACTC TGTATGCTAT ATTTCAACAG AGCAGGGGCC AAGAACAAGA
       AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT

25601  GCTGAAAATA AAAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT
       CGACTTTTAT TTTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651  ATCACAAAAG CGAAGATCAG CTTCGGCGCA CGCTGGAAGA CGCGGAGGCT
       TAGTGTTTTC GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701  CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT
       GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCCTGATCA AAGCGCGGGA

25751  TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG
       AAGAGTTTAA ATTCGCGCTT TTGATGCAGT AGAGGTCGCC GGTGTGGGCC

25801  CGCCAGCACC TGTTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC
       GCGGTCGTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGCGGG

25851  TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCA
       ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901  AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT
       TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951  CCCGGGTCAA CGGAATACGC GCCCACCGAA ACCGAATTCT CCTGGAACAG
       GGGCCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001  GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC
       CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051  CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC
       GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101  CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAACTCAGG GGCGCAGCTT
       GGTCTCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151  GCGGGCGGCT TTCGTCACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA
       CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201  CCTGACAATC AGAGGGCGAG GTATTCAGCT CAACGACGAG TCGGTGAGCT
       GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251  CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC
       GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301  CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAACTCTGC AGACCTCGTC
       GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351  CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT
       GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA

26401  TTGTGCCATC GGTCTACTTT AACCCCTTCT CGGGACCTCC CGGCCACTAT
       AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA

26451  CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG
       GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTTCCTGA GCCGCCTGCC
```

Figure 27 AB

26501 CTACGACTGA ATGTTAAGTG GAGAGGCAGA GCAACTGCGC CTGAAACACC
GATGCTGACT TACAATTCAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGGCCCGG CGCACGGCGT
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTTCTCACT
GGGTCGCGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAAATATA CTGGGGCTCC
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGGTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTCAACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG
ATGAGGTAGT CTTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCGTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT
ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT
TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCTG CTGTGTGCAC ATTTGCATTT
TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA
TAACAGTCGA AAAATTTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTC

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTCGCAG CTGAAGCTAA
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA
ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCCTGCTC AATACCATTC
TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAACT

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA
GCCTAAACAA GGTCAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA
CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

```
28451  TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG
       AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501  CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT
       GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA

28551  TTGCTTTACG GATTTGTCAC CCTCACGCTC ATCTGCAGCC TCATCACTGT
       AACGAAATGC CTAAACAGTG GGAGTGCGAG TAGACGTCGG AGTAGTGACA

28601  GGTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT
       CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651  ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT
       TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA

28701  AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTTCTGCT GATTATTTGC
       TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751  ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC
       TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801  ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAAA
       TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT

28851  GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC
       CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901  TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG
       ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC

28951  GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC
       CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001  TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT
       AAGGTGACGT TGTTCAACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051  CGCCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG
       GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101  AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG
       TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC

29151  CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA
       GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201  TCAAGAGCTC CAAGACATGG TTAACTTGCA CCAGTGCAAA AGGGGTATCT
       AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCCATAGA

29251  TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA
       AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCATT ATGGTGGCCT

29301  CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT
       GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTTA ACCACCAGTA
```

Figure 27AE

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29401  GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT
       CGACGTAAGT GAGTGGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451  AAGACCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA
       TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501  AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT
       TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551  TTATTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT
       AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601  CCTCCTGGCT GCAAACTTTC TCCACAATCT AAATGGAATG TCAGTTTCCT
       GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651  CCTGTTCCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG
       GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701  CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC
       GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751  GGAAACCGGT CCTCCAACTG TGCCTTTTCT TACTCCTCCC TTTGTATCCC
       CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801  CCAATGGGTT TCAAGAGAGT CCCCCTGGGG TACTCTCTTT GCGCCTATCC
       GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851  GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG
       CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTTT ACCCGTTGCC

29901  CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAAT GTAACCACTG
       GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951  TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT
       ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001  GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC
       CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GGCGGCGTGG

30051  TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA
       AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101  CCGTGCACGA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG
       GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTCAC

30151  TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA
       AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201  TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG
       ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251  GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA
       CATCGAACCC GTAACTGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT
```

Figure 27 AF

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30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC
      AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACTAAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA
      TTTGATTTCA ATGACCTCGG AACCCAAAAC TAAGTGTTCC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT
      GAATTACATC GTCCTCCTGA TTCCTAACTA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC
      TGAACTACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAAACTCAG CCCACAACTT GGATATTAAC
      ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CCAAAAAGCT
      ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTTGTTAA GGTTTTTCGA

30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA
      ACTCCAATTG GATTCGTGAC GGTTCCCCAA CTACAAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA
      ATCGGTAATT ACGTCCTCTA CCCGAACTTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTC
      TTGTGTTTAG GGGAGTTTTG TTTTTAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA
      TTTGTTCCGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACTGTCGT

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG
      GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTCGA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC
      TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG

30951 TAAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG
      ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTCAGTTTT GGCTGTTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT
      AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA
      GTTTCACGAG TAGAATAATA TTCTAAACTG CTTTTACCTC ACGATGATTT

31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA
      GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA
      GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAAACTGCC AAAAGTAACA TTGTCAGTCA
      CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT
```

Figure 27 AG

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31251  AGTTTACTTA AACGGAGACA AAACTAAACC TGTAACACTA ACCATTACAC
       TCAAATGAAT TTGCCTCTGT TTTGATTTGG ACATTGTGAT TGGTAATGTG

31301  TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG
       ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTCACG TATGAGATAC

31351  TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC
       AGTAAAAGTA CCCTGACCAG ACCGGTGTTG ATGTAATTAC TTTATAAACG

31401  CACATCCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT
       GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTTCTTATT TCTTAGCAAA

31451  GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA
       CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501  TTTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC
       AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551  CGTACCTTAA TCAAACTCAC AGAACCCTAG TATTCAACCT GCCACCTCCC
       GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601  TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCGGCTGG CCTTAAAAAG
       AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651  CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG
       GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701  TTTCCTGTCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC
       AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG

31751  AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG
       TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801  TCCAACTTGC GGTTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA
       AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT

31851  TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC
       ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTCG

31901  AGCGCGCGAA TAAACTGCTG CCGCCGCCGC TCCGTCCTGC AGGAATACAA
       TCGCGCGCTT ATTTGACGAC GGCGGCGGCG AGGCAGGACG TCCTTATGTT

31951  CATGGCAGTG GTCTCCTCAG CGATGATTCG CACCGCCCGC AGCATAAGGC
       GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001  GCCTTGTCCT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA
       CGGAACAGGA GGCCCGTGTC GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051  CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA
       GTCATTGACG TCGTGTCGTG GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101  GGCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT
       CCGCGACATA GGTTTCGAGT ACCGCCCCTG GTGTCTTGGG TGCACCGGTA

32151  CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG
       GTATGGTGTT CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC
```

Figure 27AH

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32251  CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC
       GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG

32301  AGCTGGCCAA AACCTGCCCG CCGGCTATAC ACTGCAGGGA ACCGGGACTG
       TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

32351  GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT
       CTTGTTACTG TCACCTCTCG GGTCCTGAGC ATTGGTACCT AGTAGTACGA

32401  CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC
       GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG

32451  TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGGAACAACC
       AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTTGG

32501  CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA
       GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

32551  ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT
       TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

32601  CCTCCAGTAT GGTAGCGCGG GTTTCTGTCT CAAAAGGAGG TAGACGATCC
       GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCCTCC ATCTGCTAGG

32651  CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTTG GTCGTAGTGT
       GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAAC CAGCATCACA

32701  CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCCTGAA GCAAAACCAG
       GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

32751  GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG
       CACGCCCGCA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

32801  CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC
       GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

32851  CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA
       GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

32901  ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCGTT
       TGTAGGTGGT GGCGTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA

32951  CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT
       GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA

33001  TTTTTTTATT CCAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA
       AAAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT

33051  GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAACTCTACA GCCAAAGAAC
       CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG

33101  AGATAATGGC ATTTGTAAGA TGTTGCACAA TGGCTTCCAA AAGGCAAACG
       TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC
```

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC
GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC
CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGAAGTCGG AGTTCGTCGC

33351 AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA
TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG
TTCGCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC
GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCCACA CTGATTATGA CACGCATACT
GGCGGTCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC
CGCTATATTT TACGTTCCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG
TTTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG
GAGGCCTTGG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT
GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG
ATCTTCGGAC AGAATGTTGT CCTTTTTGTT GGGAATATTC GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA
TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT

33901 AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT
TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG
GCCATTTGTG TAGTCCAACT AAGTGTAGCC AGTCACGATT TTTCGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC
GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG

Figure 27 A J

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34151  ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA
       TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT

34201  AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC
       TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251  AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA
       TCAGTGTCAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT

34301  AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
       TTTTTACTGC ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG

34351  GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCCTCAAA
       CGCTTGGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401  TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAACT
       AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA

34451  ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
       TGTTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGGA TGCAGTGGGC

34501  CCCCGTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC
       GGGGCAAGGG TGCGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

                                                  PacI
                                                --------
34551  ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
       TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACTACTA CAATTAATTC

34601  AATTCGGATC TGCGACGCGA GGCTGGATGG CCTTCCCCAT TATGATTCTT
       TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651  CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG
       GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701  GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
       CGTCCATCTA CTGCTGGTAG TCCCTGTCGA AGTTCCGGTC GTTTTCCGGT

34751  GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
       CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG

34801  CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
       GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851  GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
       CTGTCCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG

34901  GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
       CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG

34951  CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
       GGAAGCCCTT CGCACCGCGA AAGAGTATCG AGTGCGACAT CCATAGAGTC

35001  TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG
       AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC
```

Figure 27 AK

AAGTCGGGCT GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC
TTGGTGGCGA CCATCGCCAC CAAAAAAACA AACGTTCGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT
CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA
TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
CACATCTATT GATGCTATGC CCTCCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA
TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTCGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG

Figure 27AL

```
36051  AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
       TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101  GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA CGGGATAATA
       CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151  CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT
       GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201  TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
       AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA

36251  GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
       CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301  GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
       CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTTCCCT

36351  ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA
       TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401  TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
       AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451  AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
       TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501  AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA
       TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551  TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA
       ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

                          PacI
                        ~~~~~~~~
36601  ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
       TAAGAATTAA AGAATTAATT (SEQ ID NO:35)
```

Figure 27AM

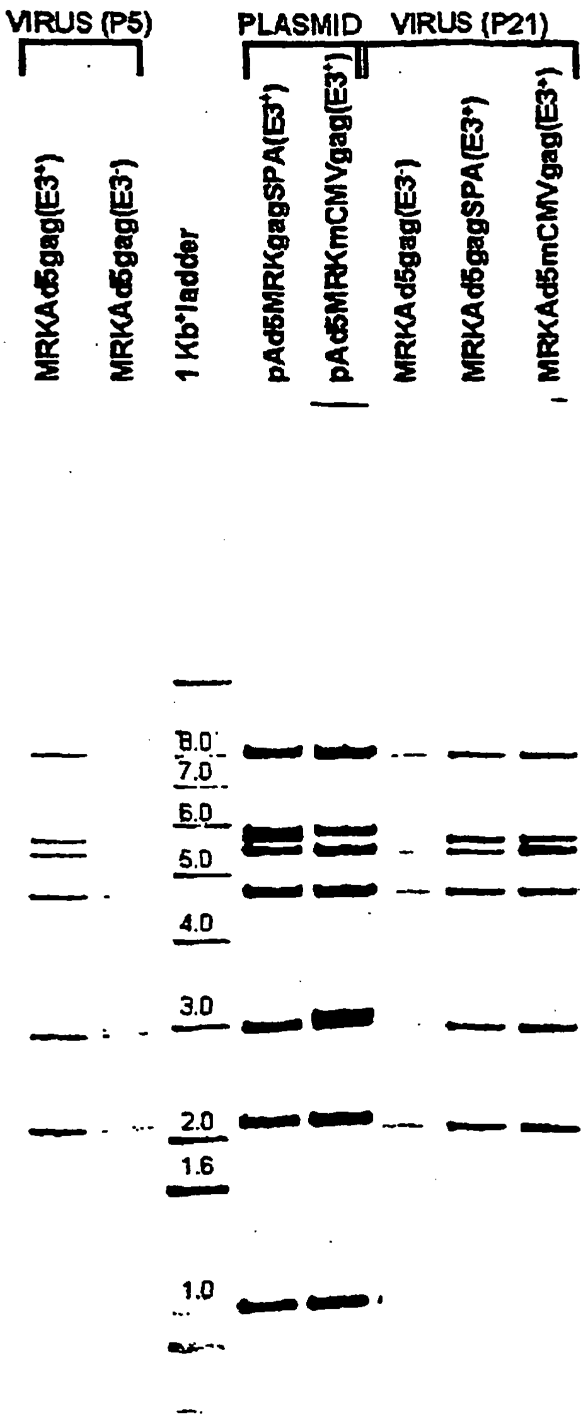

VIRUS (P5)
PLASMID
VIRUS (P21)
MRKAd5gag(E3+)
MRKAd5gag(E3-)
1 Kb+ladder
pAd5MRKgagSPA(E3+)
pAd5MRKmCMVgag(E3+)
MRKAd5gag(E3-)
MRKAd5gagSPA(E3+)
MRKAd5mCMVgag(E3+)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.6
1.0

FIGURE 28

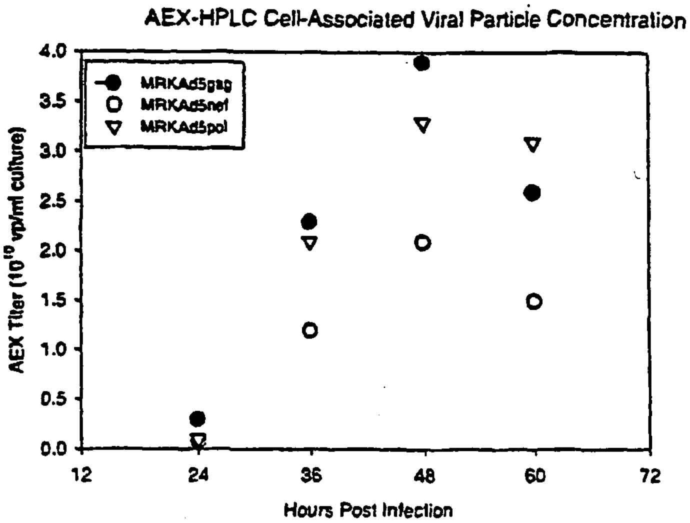


FIGURE 29A

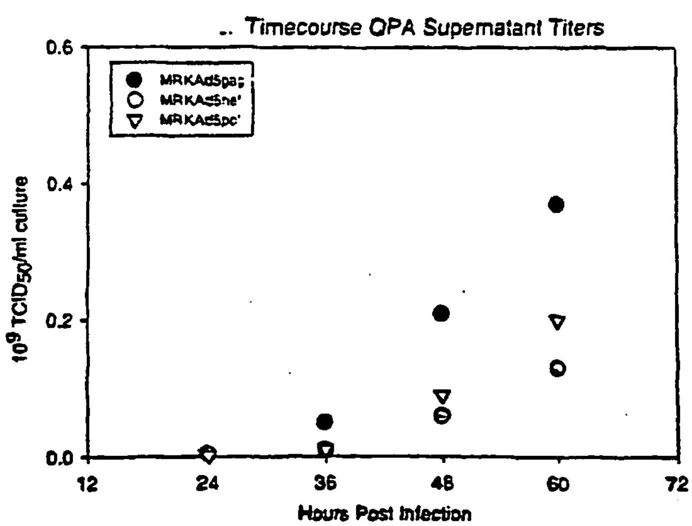


FIGURE 29B

```
atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga      48
Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1               5                   10                  15

gca gtc ttc gtt tcg ccc agc gag atc tcc att gtg tgg gcc tcc agg      96
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg
            20                  25                  30

gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag     144
Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu
        35                  40                  45

ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc     192
Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly
    50                  55                  60

tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt     240
Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys
65                  70                  75                  80

gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag     288
Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys
                85                  90                  95

att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct     336
Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala
            100                 105                 110

gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg     384
Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val
        115                 120                 125

cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc     432
Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr
    130                 135                 140

ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag     480
Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu
145                 150                 155                 160

gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac     528
Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp
                165                 170                 175

ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag     576
Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln
            180                 185                 190

atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg     624
Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu
        195                 200                 205

cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc     672
His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro
    210                 215                 220

agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att     720
Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile
225                 230                 235                 240

ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag     768
Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys
                245                 250                 255
```

Figure 30A

```
agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc      816
Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro
        260                 265                 270

acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac      864
Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp
    275                 280                 285

tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag      912
Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln
    290                 295                 300

gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac      960
Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn
305                 310                 315                 320

cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg     1008
Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu
                325                 330                 335

gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag     1056
Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys
            340                 345                 350

gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc     1104
Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr
        355                 360                 365

atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag     1152
Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys
    370                 375                 380

tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc     1200
Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala
385                 390                 395                 400

ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg     1248
Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met
                405                 410                 415

aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc     1296
Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro
            420                 425                 430

tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc     1344
Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro
        435                 440                 445

aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc     1392
Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr
    450                 455                 460

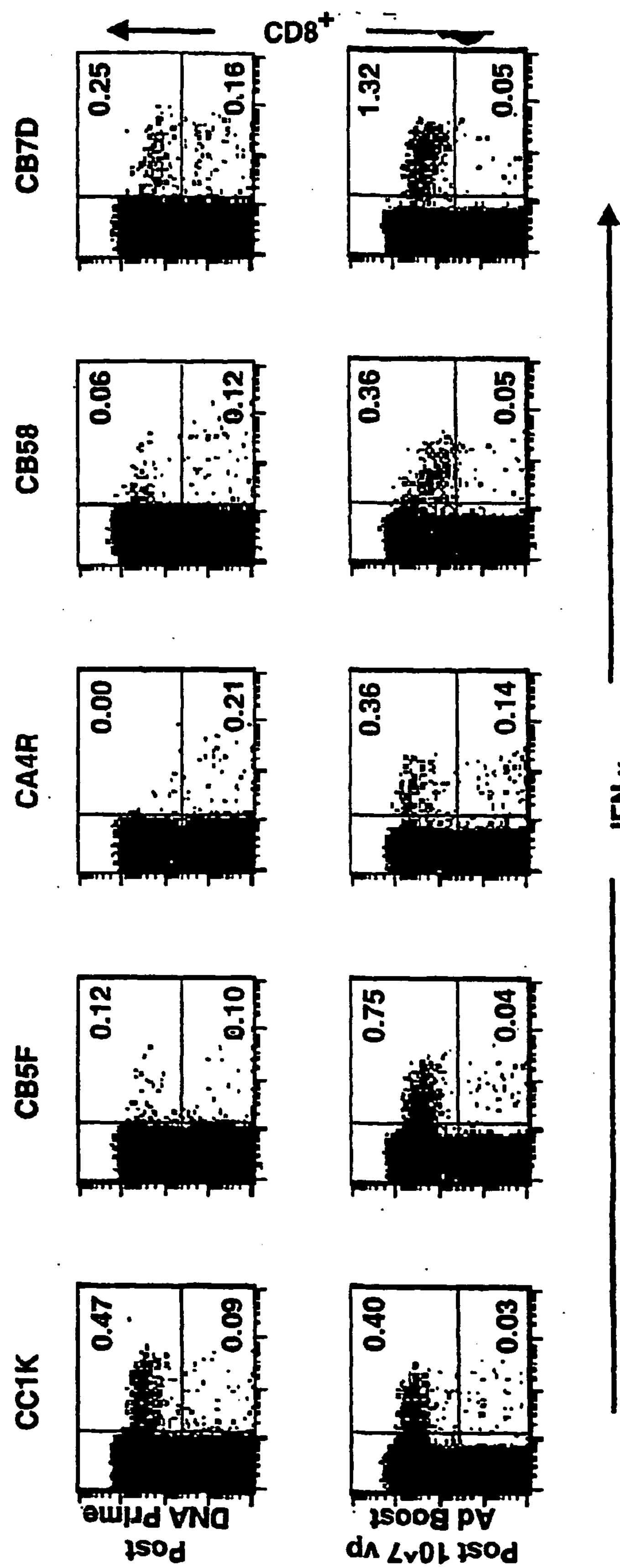
ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc     1440
Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala
465                 470                 475                 480

tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482
Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln  *  (SID NO:37)
                485                 490
```

Figure 30 B

Figure 31

IFN-γ Secretion against Gag 20-aa pool from CD3+ T cells of Monkey PBMCs



Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost Immunizations

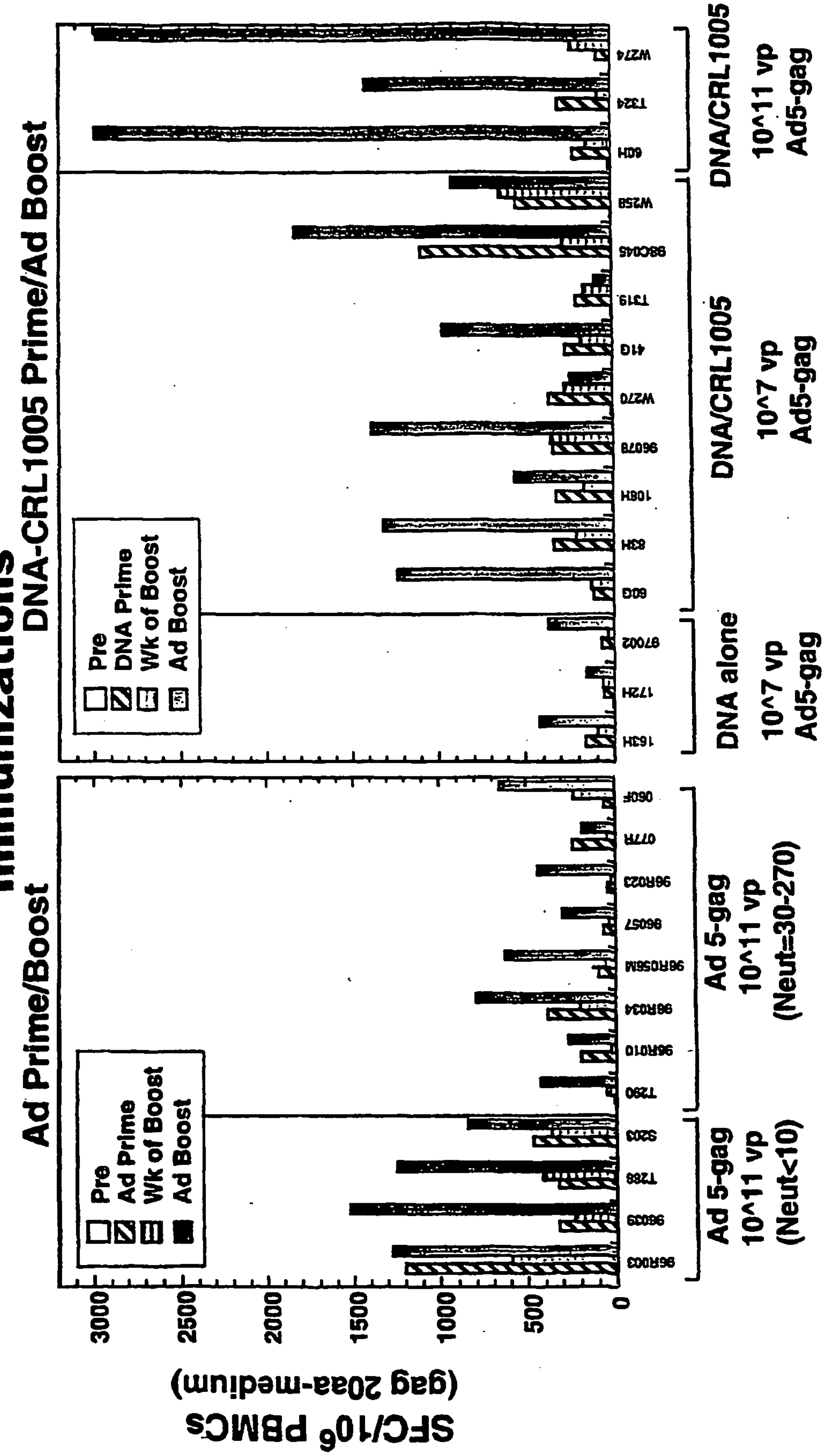


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG
CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG
CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC
CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC
ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC
CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCCAGCA GGCTGCTGCT
GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC
CAGATGGTGC ACCAGGCCAT CTCCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG
GAGAAGGCCT TCTCCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC
CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG
CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT
GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC
TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCCATCCC TGTGGGGGAA
ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC
TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTC
TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC
CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT
GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC
AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG
GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC
ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC
CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC
CACAAGGGCA GGCCTGGCAA CTTCCTCCAG TCCAGGCCTG AGCCCACAGC CCCTCCCGAG
GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC
AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCCAG
ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC
CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC
ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC
CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG
GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT
GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG
CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG
ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC
ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC
AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC
AAGAAGCACC AGAAGGAGCC CCCCTTCCTG TGGATGGGCT ATGAGCTGCA CCCCGACAAG
TGGACTGTGC AGCCCATTGT GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG
AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG
GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG
GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG
GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG
GCTGTGTTCA TCCACAACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACTC TGACATCAAG
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA
SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys
Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp
Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser
Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln
Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln
Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser
Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys
Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His
Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser
Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn
Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly
Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala
Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala
Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met
Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly
Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp
Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn
Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln
Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu
Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu
Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile
Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys
Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys
Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr
Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu
Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr
Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr
Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met
Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln
Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr
Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp
Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp
SEQ ID NO: 39

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 15/86
US CL : 435/456
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6,10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18
Y		4, 5, 13-17, 29-32, 34, 35, 37
X	US 6,019,978 A (ERTL et al.) 1 February 2000,(01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18
Y		4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18
Y		4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 February 2002 (06.02.2002)	19 AUG 2002

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Ulrike Winkler, Ph.D. Della Collins for
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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1

		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3, the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE1 and ΔE3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from two individual vectors, one expressing *nef-pol* fusion and one expressing *gag*.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from two individual vectors, one expressing *gag-pol* fusion and one expressing *nef*.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from two individual vectors, one expressing *nef-gag* fusion and one expressing *pol*.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein *gag*, *pol* and *nef* are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein *gag* and *pol* are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein *pol* and *nef* are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein *nef* and *gag* are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.0, STN-BIOSIS, MEDLINE
adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter